Extended antibiotic treatment of persistent bovine mastitis during lactation

Efficacy, economics and social influences

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Verlengde antibioticum behandeling van persistente mastitis van melkvee tijdens de lactatie

Werkzaamheid, economie en sociale invloeden

(met een samenvatting in het Nederlands)

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Contents

CHAPTER 1

General introduction

INTRODUCTION

Mastitis is an inflammation of the udder. The inflammation is either visible (clinical mastitis) or invisible (subclinical mastitis), and is caused by the immune system responding to an intramammary infection (**IMI**) of bacteria that have invaded the udder through the teat canal. Some bacteria can cause a persistent IMI of long duration while others typically cause a short, transient infection. Mastitis can be a painful disease, potentially affecting animal welfare and milk quality. Mastitis has economic consequences too, because it directly affects milk production, the primary source of income for dairy farmers. Additional costs are due to increased culling, treatment and discarded milk (Halasa et al., 2007). These costs are often underestimated by farmers (Huijps et al., 2008). In their perception, mastitis is primarily an annoying disease disturbing the daily milking routine and is often associated with uncertainty whether the cow will ever return to full production (Jansen and Lam, 2012).

As with every disease, prevention is preferred over cure and thus, the primary focus of mastitis management should be on preventive measures. Because mastitis is a multifactorial disease, the causes can be complex. Generally, mastitis control can be effective by taking the right preventive measures, as indicated in numerous papers describing cause and effect relationships between risk factors and disease occurrence (Neave et al., 1969, Munoz et al., 2007). Although improved management can successfully control mastitis, eradication of the disease is impossible because dairy cows live in an environment with large amounts of bacteria for which the udder is an ideal environment to thrive. Eventually, some quarters will be infected, and as a result, clinical mastitis may occur and this disease has to be treated. Thus, despite control efforts, treatment of mastitis is unavoidable on most if not all dairy farms.

Persistent infections

Many different bacterial species are able to cause mastitis. The initial interaction of the invading pathogen with the host immune-system, the innate immune response, is important for the outcome of infection. Mediators of inflammation are known to play critical roles in the innate immune response to IMI (Bannerman, 2009). Some bacterial species, such as *Escherichia coli*, usually show a short transient pattern (de Haas et al., 2004, Schukken et al., 2011). However, dependent on the individual host immune response (Burvenich et al., 2003), *E. coli* may also cause an acute, life threatening, clinical inflammatory response. *Staphylococcus aureus* is typically able to avoid an acute inflammatory response because it does not induce the release of major pro-inflammatory cytokines (Bannerman, 2009). Invasion with *Staph. aureus* usually results in persistent (sub)clinical IMI that stay in the udder for a long time through adherence to epithelial cells, intracellular survival and the formation of micro-abscesses, protecting them from elimination by the immune system and antibiotics (Kerro Dego et al., 2002). The innate immune response of cows to *Streptococcus uberis* seems to be typically somewhere in between *E. coli* and *Staph. aureus* in the sense that this pathogen often causes a delayed but acute inflammatory response (Bannerman, 2009) and a subsequent persistent (sub)clinical infection (de Haas et al., 2004).

Although clear differences between bacterial species exist in their ability to cause persistent infections, this is not a black and white story. Within bacterial species, strains differ in their ability to cause persistent infection (Haveri et al., 2007). For example, *E. coli* usually causes transient infections, but some bacterial strains appear more cow adapted and cause persistent infections (Döpfer et al., 1999, Bradley and Green, 2001, Dogan et al., 2006).

An acute clinical inflammatory response to invading mastitis pathogens such as *E. coli,* may be a concern for dairy farmers as it can a be a life threatening condition for the cow. Persistent (sub)clinical infections such as *Staph. aureus* and *Strep. uberis* can be a concern too, as during the long time span they remain in the udder, they become more difficult to cure, temporarily flare-up to clinical mastitis and become a potential source of infection for herd mates. For cases of persistent mastitis, farmers eventually have the choice to either treat the infected quarter with antibiotics, or to remove the cow from the herd.

When antibiotic treatment is considered, the bacteriological cure has been reported to generally be much higher for *E. coli* (70-80%, Pyörälä and Pyörälä et al., 1998) and *Strep. uberis* (50-100%, St. Rose et al., 2003, Oliver et al., 2004a) than for *Staph. aureus* (29-52%, Pyörälä and Pyörälä et al., 1998, Sol et al., 2000). If treatment is delayed, allowing the duration of infection to increase, treatment success seems to deteriorate (van den Borne et al., 2010). Therefore, the probability of treatment success of mastitis seems to be dependent on many factors, such as the cow, the bacterial species, the specific strain, the associated immune response, and the duration of infection.

Mastitis treatment

Mastitis, generally is an inflammatory response to a bacterial infection, and thus is usually treated with antibiotics. Traditionally, antibiotic containing tubes are infused intramammary because it is easy to apply after milking and high antibiotic concentrations can be reached at the site of infection with minimum use of antibiotics (Hillerton and Kliem, 2002). Additional parenteral treatment is sometimes advocated to increase cure rates of IMI (Ziv, 1980, Ziv and Storper, 1985, Owens et al., 1988, Shpigel et al., 1997) and to cure or prevent bacteremia (Wenz et al., 2001, Erskine et al., 2002). Basically, the goal of treatment is to maximize bacteriological cure because removing causative bacteria is the best guarantee to restore the physiology of the affected quarter. However, other aspects of antibiotic treatment, such as its impact on transmission dynamics, economics and prudent use have to be considered too. From an epidemiological perspective, effective treatment is important for prevention of recurrence of disease and spreading of pathogens to herd mates via the milking machine, the milker or the cow's environment. Because dairy farming is an economic undertaking, economic optimization of treatment protocols is an important issue. Last but not least, mastitis treatment protocols should incorporate prudent use of antibiotics in order to prevent the development of antibiotic resistance of pathogens.

Progress in strategies to enhance bacteriological cure by mastitis treatment has been limited in the last decades. This may be due to the difficulty to unravel the complex interaction of all the factors that determine cure; the cow (Sol et al., 1994, 1997, Dingwell et al., 2003, Østerås et al.,

1999, DeLuyker et al., 2005), the pathogen (Bradley and Green, 2009), the antibiotic (Shpigel et al., 1997, Bradley and Green, 2009) including the application route (Wenz et al., 2001, Hillerton and Kliem, 2002) and duration of treatment (Oliver et al., 2004a, b, Milne et al., 2005, Krömker et al., 2010, Jarp et al., 1989, Pyörälä and Pyörälä, 1998, DeLuyker et al., 2005). All these factors contribute to treatment success and make the outcome of treatment difficult to predict.

Clinical mastitis treatment

Mastitis is defined as clinical mastitis when the milk aspect has changed and/or the udder is red or swollen. In severe cases, the cow is also affected, has fever and is depressed. Clinical mastitis often occurs unexpectedly and it is a challenge for farmers, veterinarians and researchers to find enhance bacteriological cure by mastitis treatment has been limited in the last decades. This may be due to the difficulty to unravel the complex interaction of all the factors that determine cure; the cow (Sol et al., 1994, 1997, Dingwell et al., 2003, Østerås et al., 1999, DeLuyker et al., 2005), the pathogen (Bradley and Green, 2009), the antibiotic (Shpigel et al., 1997, Bradley and Green, 2009) including the application route (Wenz et al., 2001, Hillerton and Kliem, 2002) and duration of treatment (Oliver et al., 2004a, b, Milne et al., 2005, Krömker et al., 2010, Jarp et al., 1989, Pyörälä and Pyörälä, 1998, DeLuyker et al., 2005). All these factors contribute to treatment success and make the outcome of treatment difficult to predict.

The most effective treatment strategy in order to cure the cow as soon as possible. In some farms clinical mastitis occurs as isolated cases, most cows have only one case per lactation, where on other farms relatively few cows have frequent recurrent cases of clinical mastitis. In the latter situation, clinical mastitis cases may be recurrent flare-ups of persistent subclinical IMI. This is probably why on farms with a high Bulk Milk Somatic Cell count (BMSCC), clinical mastitis cases are usually Gram positive bacteria, such as *Staph. aureus* or *Strep. uberis*, and are likely to be the same infections that cause the rise in SCC related to subclinical mastitis in these herds (Lam, 1997, Barkema et al., 1998). Although *E. coli* mastitis is often considered to be a transient, self-limiting infection, treatment may be necessary (Suojala et al., 2013).To date, however, the efficacy of antibiotic treatment of persistent *E. coli* IMI has not been reported. Irrespective of the underlying pathogen, persistent IMI with recurrent clinical flare-ups, are a source of infection in the herd and they should either be treated successfully or removed. To improve cure rates several approaches have been advocated. One is to select the right cow for treatment, as it has been clearly shown that cow factors determine cure (Sol et al., 2000). Others are additional parenteral treatment (Ziv and Storper, 1985, Owens et al.,1988, Shpigel et al., 1997) or extending the duration of treatment.

Subclinical mastitis treatment

Subclinical mastitis is not visible because the milk, the udder and the cow appear normal. The disease can only be diagnosed by additional testing, generally done by counting the amount of somatic cells. Usually SCC > 200.000 cells/ml is considered to be an indication of an IMI (Schepers et al., 1997, Schukken et al., 2003). *Staphylococcus aureus* and *Strep. uberis* are among the

most frequently isolated subclinical mastitis pathogens (Piepers et al., 2007, Sampimon et al., 2009). Contrary to clinical mastitis, treatment of subclinical mastitis during lactation is usually not performed. This may be due to the general perception that treatment of subclinical mastitis has low cure rates. However, it is often overlooked that cure rates can be greatly improved when applied in the early stages of infection (Van den Borne et al., 2010), when selecting the right cow based on known predictive factors for cure (Sol et al., 1997) and when extending treatment duration (Oliver et al., 2004a, DeLuyker et al., 2005).

In addition to expected low cure rates, a lack of urgency for treatment during lactation results in delaying treatment until dry off (Hillerton and Berry, 2003). This delay, however, leaves a window of opportunity for pathogens to flare-up to clinical mastitis and to spread to herd mates. Studies have shown that treatment during lactation not only has a direct effect (bacteriological cure), but also has an indirect effect by preventing the occurrence of clinical flare-ups as well as preventing transmission of IMI (St. Rose et al., 2003, Barlow et al., 2009, 2013, Zadoks et al., 2001, 2003).

The decision to treat or not to treat subclinical mastitis should also be based on economic considerations. Studies on economics of lactational subclinical mastitis treatment, however, are lacking, except for *Streptococcus agalactiae* (Yamagata et al., 1987). Including the above described indirect effects and improved cure rates through early treatment of the right cow and the right treatment protocol, may well tip the balance of economics of treatment of subclinical mastitis during lactation and turn it into an economically profitable management measure.

Extended treatment

The approved duration of treatment of registered intramammary tubes marketed in the EU, usually is 1-2 days. However, a few intramammary tubes are currently being marketed with extended treatment claims for subclinical mastitis in the EU (Pirsue®, Zoetis) or with flexible dosing regimens for the treatment of clinical mastitis in the EU (Synulox, Tetra-Delta®, Zoetis) and in the US (Spectramast® LC, Zoetis).

Despite label claims generally allowing 1-2 day treatment regimes, feedback from the field suggests clinical mastitis treatment is often repeated after an initial treatment as indicated on the label, thereby extending the duration of treatment. Reports on the magnitude of extended treatment of clinical mastitis are not available because not all farmers record treatment. Nowadays, dairy farmers in The Netherlands are obliged to keep written records of all antibiotic treatments, including duration of treatment, making it possible to quantify the incidence of extended treatment at the farm and at the cow level.

If farmers and veterinarians report frequent use of extended mastitis treatment, the question arises whether it is efficacious and evidence based. Almost all peer reviewed studies designed to evaluate the efficacy of extended duration of mastitis treatment reported an increase of bacteriological cure. Nine studies evaluated clinical mastitis, 6 studies evaluated subclinical mastitis, of which only 2 studies evaluated persistent mastitis (Table 1). Papers on efficacy of extended treatment of clinical *Staph. aureus* mastitis are not abundant (n=4), and show a

beneficial effect of extended treatment for β-lactamase negative *Staph. aureus* (Jarp et al., 1989, Sol et al., 2000), a non-significant positive trend (Pyörälä and Pyörälä, 1998) or a positive significant difference (Truchetti et al., 2014). The peer reviewed papers on extended treatment of subclinical *Staph. aureus* mastitis both show a significant improvement of cure after extended treatment (Table 1). The evidence of a beneficial effect of extended treatment of clinical and subclinical *Strep. uberis* and/or *Streptococcus dysgalactiae* mastitis, is limited to 6 papers, of which 2 are from experimental trials (Table 1). Studies reporting on bacteriological cure across pathogens are limited (n=4), only 1 recent study showing no effect of extended treatment.

The focus of this thesis is on extended antibiotic treatment of persistent mastitis because, as mentioned, this type of IMI are generally considered to be difficult to cure, with a possible positive effect of extended treatment. Studies in this field are scarce (n=2, Table 1), both showing an improved bacteriological cure after extended treatment.

Table 1. Literature overview of the reported bacteriological efficacy of extended antibiotic treatment of clinical, subclinical and persistent mastitis.

Clinical mastitis	*Staph. aureus*	*Strep. uberis / dysgalactiae*	Across pathogens
Jarp et al., 1989	++[1]		
Pyörälä and Pyörälä,1998	+		
Sol et al., 2000	++[1]		
Oliver et al., 2004a		++[2]	
Krömker et al., 2010		++	++
Truchetti et al., 2014	++		++
McDougall et al., 2014			00
Subclinical mastitis			
Gillespie et al., 2002	++	+	
Oliver et al., 2003		++[2]	
Oliver et al., 2004b		++	
Roy et al., 2009	++		
Steele and McDougall, 2014			++
Persistent mastitis			
Milne et al., 2005 (clinical)		++	++
DeLuyker et al., 2005 (subclinical)	++		

[1] only for β-lactam negative strains, [2] experimental infection, ++ significant difference ($p<0.05$), + trend ($p<0.2$), 00 no difference ($p>0.2$)

Social influence on clinical mastitis treatment

Antibiotic treatment of mastitis is often extended, but this is not always evidence based. Therefore, the question arises why farmers do it. Who influences farmers' decision making, their opinion and mindset in this field and how does this occur? Who are the major actors in their social world (Leeuwis and van den Ban, 2004) influencing antibiotic mastitis treatment in general

and extended treatment specifically? How do farmers see the value of antibiotics and necessity of judicious use, and how do they adapt their practices to these perceptions? Although Jansen et al. (2010) identified the most important information sources for farmers, few authors have looked at the social influence on antibiotic use decision-making. Exploring social relations and their perceived social pressure on antibiotic mastitis treatment practices by dairy farmers may be valuable in order to understand their behavior and may eventually reveal tools to change behavior, if such a change is deemed necessary.

Aim and scope of the thesis

In this thesis, extended treatment of persistent mastitis during lactation is explored. This was done by studying the bacteriological, clinical and economic effects of extended mastitis treatment during lactation as compared to standard treatment. In addition, social influence on the choices underlying antibiotic treatment of mastitis was studied.

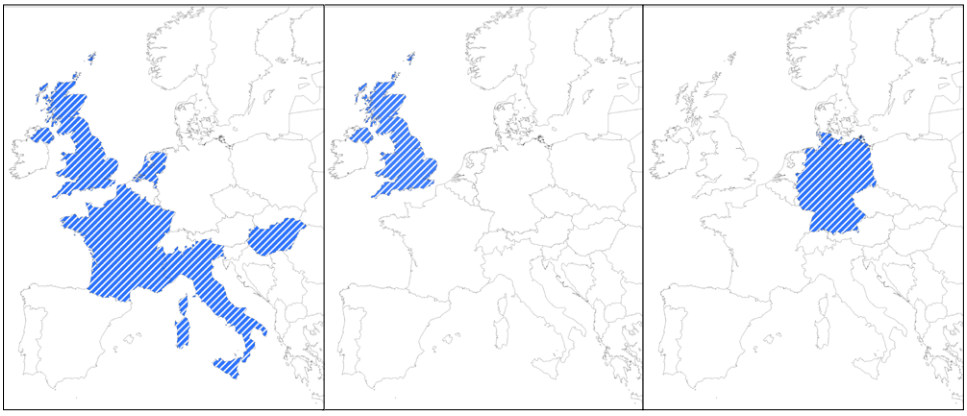
The economic effect of lactational persistent subclinical mastitis treatment was evaluated in Chapter 2 and 3. The aim of the studies described in these chapters was to explore whether lactational treatment of subclinical mastitis is economically profitable when indirect effects of treatment, prevention of clinical flare-ups and transmission of IMI to herd mates is included in the calculations. In these studies the identification of the most appropriate treatment candidates, and the impact of treatment duration on the economics of treatment and cure were also included. The efficacy of extended treatment of persistent clinical mastitis was evaluated in Chapter 4, 5 and 6. The aim of the studies described in these chapters was primarily to investigate bacteriological and clinical efficacy of extended treatment of persistent clinical mastitis and compare it to standard, label, treatment. To study different aspects of the efficacy of extended duration of antibiotic treatment of clinical mastitis, multiple trials in a number of European countries were performed. These trials included:

1) Seventy-seven farms in 5 different EU countries with clinical mastitis cases caused by S*taph. aureus* (Chapter 4)
2) Three farms in Somerset, UK, with predominantly persistent recurrent *E. coli* infections (Chapter 5)
3) Twenty farms in Germany, where treatment of clinical mastitis cases preceded by 2 consecutive monthly cow SCC over 200.000 cells/ml were evaluated (Chapter 6)

The geographical distribution of the 3 clinical trials is shown in Figure 1.

Social influences of antibiotic use for mastitis treatment were evaluated in Chapter 7. The aim of that study was to investigate by whom and how dairy farmers are influenced in their social environment in decision making related to (extended) antibiotic mastitis treatment. Semi-structured interviews of 17 dairy farmers in The Netherlands and 21 in Germany formed the basis to evaluate these questions.

The aim of this thesis is to quantify bacteriological, clinical and economic efficacy of extended antibiotic treatment as compared to standard treatment of persistent bovine mastitis during lactation. A second aim was to explore the social factors that influence farmers with respect to their decisions on the duration of antibiotic treatment of mastitis.



Study 1 Study 2 Study 3

Figure 1. Overview of the geographical distribution of 3 different clinical trials exploring the efficacy of extended treatment of persistent clinical mastitis. Study 1; extended treatment of clinical *Staph. aureus* mastitis (UK, NL, I, Hu, F), Study 2; extended treatment of recurrent environmental clinical mastitis (UK), and Study 3; extended treatment of (persistent) clinical mastitis (De).

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CHAPTER 2

Use of partial budgeting to determine the economic benefits of antibiotic treatment of chronic subclinical mastitis caused by *Streptococcus uberis* or *Streptococcus dysgalactiae*

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ABSTRACT

The economic effect of lactational antibiotic treatment of chronic subclinical intramammary infections due to *Streptococcus uberis* or *Streptococcus dysgalactiae* was explored by means of partial budgeting. Effects at cow level and herd level were modelled, including prevention of clinical mastitis episodes and the prevention of transmission of infections. Input variables for our deterministic model were derived from literature or based on 2002/2003 dairy prices and farming conditions in The Netherlands. Sensitivity analysis was used to examine the effect of uncertainty around input variables or changes in price estimates. On farms where pathogen transmission was prevented through proper udder health management, 3-d antibiotic treatment during lactation resulted in an average net profit of €+11.62 over no treatment while 8-d antibiotic treatment had an average negative net result of €–21.83. Sensitivity analysis showed that profitability depends on the probability of treatment-induced cure, pathogen transmission rates, culling rate, retention pay-off, and costs of antibiotic treatment. Three-day antibiotic treatment of chronic subclinical streptococcal mastitis is economically profitable over a range of input values for cure probabilities, transmission rates and losses due to culling. In contrast, 8-d lactational treatment is only profitable for very valuable animals, on farms where the risk of pathogen transmission is high and/or when the farmer is likely to cull a high percentage of cows with subclinical mastitis. Because bacterial flora, cow characteristics and management differ widely between farms, the economic outcome of lactational treatment of chronic subclinical streptococcal mastitis may be highly farm-dependent.

Keywords: Economics, antibiotic treatment, subclinical mastitis, *Streptococcus uberis*, *Streptococcus dysgalactiae*.

INTRODUCTION

Mastitis is the most costly disease in dairy cattle in developed countries (Smith & Hogan, 2001). Costs are mainly due to milk production losses, culling, treatment and milk discarded because of antibiotic residues (Craven, 1987; Esslemont & Kossaibati, 1997; Hortet & Seegers, 1998). Additional costs include decreased fertility (Schrick et al. 2001), changed composition of milk (Hortet & Seegers, 1998), and risk of violation of bulk tank limits or loss of premium for low bulk tank somatic cell count (BTSCC) (Allore et al. 1998; Hogeveen, 2003). In cases of clinical mastitis, farmers are usually willing to treat animals because the animals are diseased, milk is visibly abnormal, and/or milk production has decreased dramatically. Treatment of clinical mastitis is not only a matter of cost v. benefit. Legal, ethical and animal welfare arguments also need to be considered in treatment decisions. For example, according to Milk Hygiene directive 92/46 EEC it is not allowed to deliver milk from cows suffering from recognizable inflammation of the udder. In cases of subclinical mastitis, animals are not clinically diseased and milk is not visibly abnormal. Therefore, inflammation is not recognizable without additional testing and treatment may not seem necessary. Subclinical mastitis, like clinical mastitis, affects milk quality and quantity, and is associated with economic losses as described above. Furthermore, cows with subclinical infections may act as a source of infection for other animals, resulting in spread of a mastitis problem in the herd. If benefits of treatment of subclinical mastitis outweigh the costs, treatment may be economically viable, especially when milk quality is a significant component of price (Hillerton & Berry, 2003) or when clinical cases (St. Rose et al. 2003) or infection transmission (Zadoks et al. 2002) can be prevented.

Treatment of subclinical mastitis is often deferred until the dry period (Hillerton & Berry, 2003). However, treatment of subclinical *Streptococcus agalactiae* infections during lactation is economically profitable (Yamagata et al. 1987). The success of treatment programmes for *Str. Agalactiae* is partly due to the high proportion of quarters cured after treatment, and to the prevention of disease transmission that is achieved through cure of infected animals (Loeffler et al. 1995). Reported cure proportions for *Streptococcus uberis* and *Streptococcus dysgalactiae* are high too, ranging from 50% to 100% (Bramley, 1984; Owens et al. 1997; McDougall, 1998). Recent studies have shown that treatment of subclinical infections with non-agalactiae streptococci may contribute to prevention of clinical mastitis (St. Rose et al. 2003) and to prevention of streptococcal transmission (Zadoks et al. 2001a, 2003). The cost-benefit ratio of antibiotic treatment of subclinical *Str. uberis* and *Str. dysgalactiae* infections during lactation has not been determined.

The purpose of this paper is to explore the economic benefit of antibiotic treatment of chronic subclinical *Str. uberis* and *Str. dysgalactiae* infections during lactation by means of partial budgeting. In this analysis, effects at the cow level, such as bacteriological cure and prevention of clinical mastitis, and effects at herd level, such as reduced transmission potential, will be taken into account.

MATERIAL AND METHODS

Partial budgeting was used for the development of a deterministic simulation model to estimate the net cost or benefit of lactational treatment of subclinical streptococcal mastitis with antibiotics. The model was specifically adapted for two mastitis-causing agents, *Str. uberis* and *Str. dysgalactiae*, because they are highly prevalent pathogens in many dairy countries but, unlike for *Str. agalactiae*, the economic feasibility of treatment of infections has not been explored. Input variables for the model were based on literature, if available, or on the 2002/2003 dairy situation and prices in the Netherlands. Costs and benefits were calculated at the cow level during one lactation. Three treatment scenarios were explored: no treatment, 3-d treatment (St. Rose et al. 2003), and 8-d treatment (DeLuyker et al. 2001). The choice of treatment duration was based on common practice and availability of registered antibiotics for parenteral (3-d) or intramammary (8-d) treatment of subclinical mastitis in the Netherlands. For each of the three treatment scenarios, a sensitivity analysis was performed to determine the impact of input variables, including the probability of cow-to-cow transmission of mastitis pathogens. Four scenarios were analysed, i.e., 3-d treatment and 8-d treatment, combined with the transmission scenario with low risk of contagious transmission ($R < 1$, specifically $R = 0.21$) or high risk of contagious transmission ($R > 1$, specifically, $R = 1.4$). In each scenario, sensitivity analysis was performed for all input variables that are listed in Table 1.

A schematic outline of the deterministic model is depicted in Fig. 1, and details of input variables and model assumptions are presented below.

Model Inputs

Probability of Cure

Under Dutch farming and screening conditions, subclinical mastitis is suspected if two out of three consecutive milk samples taken at 3-week or 4-week intervals have somatic cell counts (SCC) > 250,000 cells/ml. Thus, a subclinical streptococcal infection would have been present for at least 3 or 4 weeks before it was eligible for treatment. Usually, a decision to treat will be preceded by milk sample collection and bacteriological culture, adding to the duration of infection before treatment, if any, is initiated. The probability of spontaneous bacteriological cure for chronic subclinical streptococcal infections was estimated to be between 0% (St. Rose et al. 2003) and 20.5% (DeLuyker et al. 2001: 25% for *Str. uberis*, 16% for *Str. dysgalactiae*). The arithmetic average, i.e. 10%, was used in our model (Fig. 1). After 2-d or 3-d treatment with intramammary or parenteral antibiotics, cure probabilities have been reported to be 82.6% for *Str. uberis* (McDougall, 1998), 58.6% for both species combined (St. Rose et al. 2003), and 33.5% for *Str. uberis* and 73.5% for *Str. dysgalactiae* (DeLuyker et al. 2001). The arithmetic average, 62%, was used as the estimated probability of cure after 3-d treatment in our model (Fig. 1). After 8-d treatment, the probability of cure is 75% for *Str. uberis* and 100% for *Str. dysgalactiae* (DeLuyker et al. 2001). The average, 87.5%, was used as the estimated probability of cure after 8-d treatment in our model (Fig. 1). Chronic subclinical infections that do not cure may remain subclinically infected or develop into

clinical flare-ups. The probability of clinical flare-ups is estimated at 19.3% (average of Lam, 1996: 27.5%; St. Rose et al. 2003: 16.7%; Zadoks et al. 2003: 13.8%) (Fig. 1).

Probability of Transmission

Cows with chronic streptococcal mastitis and continued bacterial shedding cause extended exposure of the whole herd to pathogenic bacteria which may result in infection of other cows in a herd (Zadoks et al. 2001a; Hillerton & Berry, 2003). The extent to which transmission to other cows occurs is species dependent and is higher for *Str. agalactiae* than for non-agalactiae streptococci, and higher *for Str. dysgalactiae* than for *Str. uberis* (Neave et al. 1969; Fox & Gay, 1993).

The infectiousness of a pathogen can be expressed in the transmission parameter, β, i.e., the average number of new infections caused by an infectious individual per unit of time. For mastitis, this would translate into the number of new infections caused per day by an infected cow (Zadoks et al. 2001a). The total number of new infections caused by an infected individual also depends on the duration, t, of the infection in that individual. The combined effect of infectiousness and duration is represented by the reproductive ratio, R, which is the total number of new infections caused by an infected individual during its infectious lifetime. R is commonly represented as β/α, where α is the cure rate. The cure rate is equivalent to 1/t, i.e., the inverse of duration.

The duration of non-agalactiae streptococcal infections has been described in several studies, and ranges from 1 d to a full lactation (Todhunter et al. 1995; Zadoks et al. 2003). Median duration has been estimated at 42 d for *Str. uberis* (Zadoks et al. 2003), while mean durations have been estimated at 13 d for environmental streptococci (Todhunter et al. 1995), at 67 d for *Str. uberis* (Zadoks et al. 2003), at 56–96 d for *Str. uberis* (Lam et al. 1997), and at 34–81 d for *Str. dysgalactiae* (Lam et al. 1997). Because duration of infection is not normally distributed, the median (42 d) is probably a more appropriate measure of duration than the mean. Therefore, we assumed $\tau = 42$ *days or* $\alpha = 0.024$ as the most likely scenario. For those animals that are eligible for and/or subjected to treatment, there is left and right censoring of the duration of infection. Animals are not considered eligible for treatment until they have been infected for at least 3–4 weeks (minimum duration for detection of two subsequent SCC values > 250,000 cells/ml, plus time needed for diagnosis and treatment decision). This censoring excludes infections of less than approximately 30 d of duration. Animals that receive treatment will cure in a large proportion of cases, resulting in right censoring of the duration of infection. Because diagnosis, treatment decision and treatment usually take several days or even weeks, the maximum duration of infection would be around 50 d for such animals. Hence, 42 d seems a reasonable estimate for duration of infection both for untreated and for treated animals.

Estimates for the transmission parameter of mastitis causing bacteria are scarce owing to the labour-intensive nature of studies needed to generate them (Lam, 1996; Zadoks et al. 2001a, 2002). The transmission parameter for *Str. uberis* has been estimated from field data as 0.033 during an outbreak of *Str. uberis* mastitis, while it was much lower, 0.005, during a non-outbreak situation, as described in detail elsewhere (Zadoks et al. 2001a).

Table 1. Partial Budgeting: calculation of net profit (€) of 3-day or 8-day lactational treatment versus no treatment of chronic subclinical mastitis due to *Streptococcus uberis* or *Streptococus dysgalactiae*. Net profit is calculated as (extra revenue + reduced costs) minus (reduced revenue + extra costs).

Contribution to Economic Effect	Reference	Treatment	
		3-day	8-day
Extra revenue:			
Increase in milk production after cure (kg milk)	McDermott, 1983 St. Rose, 2003	0	0
Total extra revenue	Calculated	0	0
Reduced costs:			
Reduction in probability of clinical mastitis after treatment (%)	St. Rose, 2003 Calculated	12.3%	17.3%
Costs of clinical flare-up of pre-existing subclinical mastitis	De Vos & Dijkhuizen,1998	117	117
Reduced costs due to prevention of clinical flare-ups (€)	Calculated	14.39	20.24
Reduction in probability of persistent subclinical mastitis (%)	Calculated	43.7%	61.7%
Number of new infections that is prevented	This paper	0.15	0.15
Probability that new infection results in spontaneous cure (%)	McDougall, 1998 Zadoks, 2003 Smith, 1985 Wilson, 1996, 1999	38%	38%
Probability that new infection results in clinical mastitis (%)	Jayarao, 1999 Lam, 1996 Zadoks, 2003	30%	30%
Probability that new infection results in chronic subclinical mastitis (%)	This paper	32%	32%
Costs of spontaneous cure (€)	This paper	5	5
Cost of clinical mastitis (€)	De Vos & Dijkhuizen,1998	209	209
Costs of subclinical mastitis (€)	This paper	122.80	122.80
Reduced costs due to prevented transmission (€)		6.81	9.62
Reduction in probability of persistent subclinical mastitis (%)		43.7%	61.7%
Retention pay off (€)	De Vos & Dijkhuizen,1998	526	526
Culled animals (%)	Esslemont & Kossaibati, 1997 Whitaker, 2001 NRS, 1998 This paper	12%	12%
Reduced costs due to prevented culling (€)		27.58	38.95
Reduced costs due to prevented penalties for high scc		0	0
Reduced costs due to prevention of decreased fertility		0	0
Total reduced costs (€):		48.78	68.80
Reduced revenue:			
Milk discard because of antibiotic residue (kg/day)	NRS, 1998	24.2	24.2
Duration of milk withhold (days)	This paper	6	11
Total discarded milk (liters)		145.2	266.2
Balanced profit milk (€/kg)		0.07	0.07
Total reduced revenue (€):		10.16	18.63
Extra costs:			
Antibiotics (€)	This paper	27	72
Labour (€)	This paper	0	0
Costs penalties antibiotic residues in milk (€)		0	0
Total extra costs (€):		27	72
Net profit (€):		+11.62	-21.83

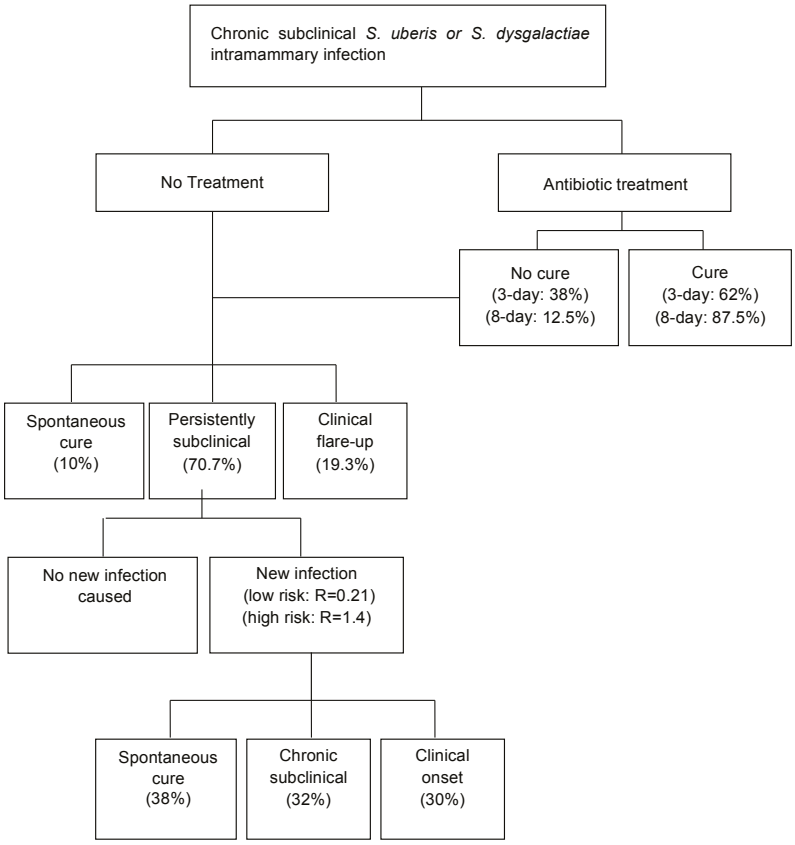

Figure 1. The deterministic model for effect of 3-day or 8-day antibiotic treatment versus no treatment of chronic subclinical intramammary infections with *Streptococcus. uberis* and *Streptococcus dysgalactiae*. Flow through diagram is from top to bottom, not in reverse. *R = reproductive ratio, i.e., the total number of new infections caused by an infected individual during its infectious lifetime (Zadoks et al. 2001a).*

Briefly, an outbreak is where many new infections occur over a short period, probably as a result of contagious transmission, while a non-outbreak is where contagious transmission is controlled and new infections originate predominantly from the environment (Zadoks et al. 2001a, 2003). In our economic model, 0.005 was used as baseline estimate for b while 0.033 was considered a worst-case scenario. Estimates were originally based on udder quarter as the unit of analysis, but were used as cow-level estimates in our economic model, for lack of a better approximation. For *Str. dysgalactiae*, no estimates of the transmission parameter are available, and values for *Str. uberis* were also applied to *Str. dysgalactiae*. Based on our estimates for duration ($\tau = 42$) and the transmission parameter ($\beta = 0.005$ with good control of contagious transmission; $\beta = 0.033$ with poor control of contagious transmission), the reproductive ratio, R, is calculated to be 0.21 or 1.4 for scenarios with good and poor control of contagious transmission, respectively (Fig. 1).

Treatment of chronic subclinical mastitis reduces the duration of infection and therefore the chance of transmission to other cows. For *Str. uberis* infections that last longer than 42 d, median duration is 72 d (original data from Zadoks et al. 2003). According to Lam et al. (1997), duration is similar for *Str. uberis* and *Str. dysgalactiae*, so one estimate for both species was used. As a result, prevented duration of infection by treatment is calculated as 30 d and hence prevented contagious transmission is estimated at 0.005 × 30 = 0.15 new infections under good herd management (Table 1), and 0.033 × 30 = 1 new infection under poor herd management.

When transmission of infection occurs, the new infection may be clinical or subclinical. For *Str. uberis*, the probability that a new infection is clinical has been reported as 48% in seven Dutch herds with annual BTSCC < 150,000 cells/ml (Lam, 1996), as 15% in two Dutch herds with annual BTSCC between 200,000 cells/ml and 300,000 cells/ml (Zadoks et al. 2003), and as 5% in one research herd in the USA (Jayarao et al. 1999). For *Str. dysgalactiae*, 51% of infections were reported to have clinical onset (Lam, 1996). The arithmetic average probability that a new infection with either species has clinical onset is 30% (Fig. 1). The remaining 70% of new infections have subclinical onset. The infections with subclinical onset may cure within a month, or become chronic so that they would be detected under the Dutch 3–4-weekly sampling scheme. During the first month of subclinical infection, spontaneous cure was observed for *Str. uberis* in 73.2% of cases in New Zealand (McDougall, 1998), and 37.5% of cases in The Netherlands (original data from Zadoks et al. 2003), and for non-agalactiae streptococci in 38.5%, 60% or 59% of cases (Smith et al. 1985, Wilson et al. 1996, Wilson et al. 1999, respectively). The arithmetic average probability of spontaneous cure based on those estimates is 54%. Thus, of all new infections, 30% are assumed to be clinical at onset, 54% of the remaining 70%, i.e., 38%, are assumed to be subclinical in onset followed by spontaneous cure within a month, and the remaining 32% of new infections are expected to become subclinical and chronic (Fig. 1). Flare-ups of chronic subclinical infections to clinical cases are incorporated in the cost calculations, but remission of clinical cases to subclinical infections is not included in the model.

Economic calculations

To enable calculation of economic effects, the input variables are divided into four parts: extra revenue, reduced costs, reduced revenue and extra costs. If the sum of extra revenue and reduced costs is larger than the sum of reduced revenue and extra costs, the net result is positive. A positive net result means treatment is economically profitable. A negative net result means treatment is not economically profitable (Dijkhuizen & Morris, 1996). Input variables used for calculation of economic effect are listed in Table 1.

Extra revenue

Increase in milk production resulting from treatment would be extra revenue. This increase was assumed to be zero based on results from McDermott et al. (1983) and St. Rose et al. (2003) who found no increase in milk production after bacteriological cure of subclinical mastitis (Table 1).

Reduced costs

Successful treatment of chronic subclinical mastitis may prevent other costs. Costs can be reduced by (1) prevention of clinical mastitis (St. Rose et al. 2003), (2) prevention of transmission of infection to other cows (Zadoks et al. 2001a), (3) prevention of culling (Esslemont & Koissabati, 1997), (4) prevention of penalties for high SCC (Allore et al. 1998; Hoogeveen, 2003), and (5) prevention of losses due to poor fertility (Schrick et al. 2001).

The estimated reduced cost due to prevention of clinical mastitis is based on results from the Netherlands as reported by De Vos & Dijkhuizen (1998), who considered clinical cases that were new infections and clinical cases that were flare-ups of pre-existing subclinical infections. For streptococci, cost of new clinical mastitis cases was calculated as €209 while the cost of clinical flare-ups of subclinical infections was calculated as €117 (De Vos & Dijkhuizen, 1998) (Table1). For both situations, costs of premature culling, antibiotic treatment and discarded milk were included in the cost estimate, while milk loss is only attributed to the clinical mastitis if it is a new infection, but not when it is part of a pre-existing infection. Without treatment, the probability of clinical flare-up is 19.3% (Fig. 1). With treatment, clinical flare-up only happens in non-cured cases (Fig. 1), i.e., in 19.3% of 38% or 7% of treated cases for 3-d treatment, and in 19.3% of 12.5% or 2% of treated cases for 8-d treatment. Thus, the reduction in probability of clinical flare-up is 19.3% –7%=12.3% and 19.3% –2%=17.3% for 3-d and 8-d treatment, respectively (Table 1). The estimated reduced cost due to prevention of subclinical mastitis can be attributed to reduced probability of persistent subclinical mastitis, to prevention of new infections, prevention of culling, and prevention of elevated BTSCC. The probability of persistent subclinical mastitis is 70.7% without treatment (Fig. 1). After treatment, 38% or 12.5% of cases do not cure (Fig. 1), and 70.7% of these non-cured cases will persist as subclinical infections. Thus, 70.7% of 38%, i.e., 27%, or 70.7% of 12.5%, i.e., 9% of treated cases will persist as chronic subclinical infection after 3-d and 8-d treatment, respectively. This results in a reduction of chronic subclinical infections of 70.7% –27%=43.7% and 70.7%–1.25%= 61.7% for 3-d and 8-d treatment, respectively (Table 1). When new subclinical infections are prevented, the milk production losses and probability of culling, clinical flare-up and transmission associated with such infections are prevented, resulting in reduced costs. Milk production losses of a cow with SCC of 50,000 cells/ml are assumed to be zero, while there is a decrease in milk production of 0.4 kg/d for heifers and 0.6 kg/d for a multiparous cow for every doubling of the cow-level SCC (Hortet & Seegers, 1998). The average SCC is $10^{6.34}$ cells/ml for quarters infected with *Str. dysgalactiae* and $10^{6.72}$ cells/ml for quarters infected with *Str. uberis* (Schepers et al. 1997). When using the geometric average for SCC of *Str. dysgalactiae* or *Str. uberis* infected quarters, and assuming that the average cow has one infected quarter (SCC = $10^{6.53}$ cells/ml) and three non-infected quarters (SCC = 50,000 cells/ml) with equal milk production per quarter, the SCC of an infected cow can be calculated to be $10^{5.95}$ cells/ml or 884,610 cells/ml, implying a greater than 16-fold increase in SCC, or a loss of 1.6 kg/d for heifers and 2.4 kg/d for multiparous cows. We assumed that the probability of a new infection was independent of stage of lactation (Zadoks et al. 2001b) and that, on average, intramammary infection starts at 150 d from calving. Under that assumption, production loss was calculated as 150 × 1.6 kg/d = 240 kg/heifer per lactation and 150 × 2.4 kg/d = 360 kg/cow per lactation, with

an arithmetic average of 300 kg. This calculation does not take into account that older cows are more likely to get new infections than heifers (Zadoks et al. 2001b), that herds are usually composed of < 50% heifers, or that infection and production losses may occur in more than one quarter per cow. The importance of errors in estimated production losses were evaluated as part of the sensitivity analysis.

A spontaneous cure of a new subclinical infection is still considered to result in production loss because we assumed the cow had been infected for a short period (approximately 30 d). This production loss is then calculated as 30 d × 1.6 kg/d = 48 kg, or 30 d × 2.4 kg/d = 72 kg. The costs of spontaneous cure are assumed to be 72 × 0.07 =€5 (Table 1). The total costs of a new subclinical infection include milk production loss, premature culling and transmission to other cows and were calculated to be €122.80 (Table 1).

Prevention of culling is another reduced cost that results from prevention of new subclinical infections. Culling due to mastitis was 10.1% and 16.3% of the total number of cows culled per year among 50 and 340 herds, respectively, in the UK (Esslemont & Kossaibati, 1997; Whitaker et al. 2001). The latter value is close to the average culling percentage (17.6% of culled cows was culled because of mastitis) reported for a limited number of research herds in the Netherlands (Smolders et al. 1994). For our model, we used an estimated culling percentage due to udder health of 15% of the total number of cows culled per year. Of the culling due to mastitis, 50–65% was attributed to subclinical mastitis. On average, 34% of cows are culled annually on Dutch farms (NRS data, 1998), so that 3% (50–65% of 15% of 34%) of the total number of cows would be culled due to subclinical mastitis. When assuming that on average approximately 25% of animals are subclinically infected in a herd with BTSCC of 200,000–300,000 cells/ml (Eberhart et al. 1982), the probability of an already subclinically infected animal being culled is 12%. For the average value of an animal we used the retention pay-off of €526 (De Vos & Dijkhuizen, 1998). In this retention pay-off, salvage value has been allowed for.

Other reduced costs, such as prevention of impaired fertility or prevention of penalties for high SCC were not considered in the current model, although it is acknowledged that penalties and bonuses in particular may be important factors in the economics of mastitis (Allore et al. 1998).

Reduced revenue

Reduced revenue of treatment is the discarded milk due to antibiotic residues resulting from treatment. Average milk production was assumed to be 24.2 kg (8073 kg in 334 d of lactation) (NRS, 1998) (Table 1). The average milk-withholding period is assumed to be 6 d for 3-d treatment and 11 d for 8-d treatment, equivalent to the duration of treatment plus an extra 3 d of milk withhold (six milkings when milking twice a day).

Extra costs

The extra costs for treatment include costs of diagnostic testing, antibiotics, and extra labour. In addition, there is a risk of penalties for antibiotic residues in the milk when cows are treated. Diagnostic testing can be done at quarter, cow or herd level, through evaluation of SCC patterns,

or through culture of milk samples from individual quarters, cows, clinical cases, or bulk tank milk. Because of the variability of methods and associated cost, we did not include the cost of diagnostic testing in our model.

Table 2. Sensitivity analysis: Effect on net profit of the five most influential input variables (proportional change in output higher than proportional change in input) in the scenario of 3-day or 8-day treatment on a farm with a low probability of contagious transmission of *Streptococcus uberis* or *Streptococcus dysgalactiae.*

	Net profit 3 days treatment (€)	**Net profit 8 days treatment (€)**
Bacteriological cure (%)		
21 (DeLuyker *et al.* 2001)	-20.56	-74.03
39 (DeLuyker *et al.* 2001)	-6.82	-60.29
50 (DeLuyker *et al.* 2001)	1.81	-51.66
59 (St. Rose *et al.* 2003)	8.88	-44.59
69 (DeLuyker *et al.* 2001)	16.73	-36.74
75 (DeLuyker *et al.* 2001)	21.04	-32.43
82 (McDougall, 1998)	27.32	-26.15
90 (Owens *et al.* 1997)	33.21	-20.26
100 (DeLuyker *et al.* 2001)	41.06	-12.41
Number of new infections caused by an existing infection (R)		
0.21 (low risk of contagious transmission) (Zadoks *et al.,* 2001a)	11.62	-21.83
1.4 (high risk of contagious transmission) (Zadoks *et al.,* 2001a)	68.60	58.62
Proportion of cows with subclinical mastitis that is culled (%)		
0	-15.96	-60.78
12 (This paper)	11.62	-21.83
20	30.01	4.13
Retention pay off (€)		
0	-15.96	-60.78
526 (De Vos & Dijkhuizen, 1998)	11.62	-21.83
1200	46,97	28.07
Antibiotic costs (€)		
15	23.62	35.17
27 (This paper; 3-day treatment)	11.62	23.17
38	0.62	10.93
50	-11.38	0.17
72 (This paper; 8-day treatment)	-33.38	-21,83

Costs of antibiotic treatment depend on the treatment regimen, i.e., choice of drug, dosage, route of administration, and treatment duration. In 2002/2003, the farmers' price in an average Dutch veterinary practice for a 3-d treatment was estimated to be €27 and for an 8-d treatment to be €72 (Table 1).

Labour for treatment has to be taken into account if the farmer can economically use the time that is saved by not treating the animal. Since this is not likely, we have assumed the labour costs to be zero. Extra costs due to penalties for antibiotic residues in milk are also neglected, because they can be prevented through good management (Table 1).

Sensitivity Analysis

Sensitivity analysis is used to calculate what happens to the net result if one input variable at a time is changed from the average situation. Input variables that have a strong effect on the return on investment need to be estimated in a herd-specific manner to give adequate economic prognoses for antibiotic treatment in specific herds. When estimates for input variables that have a strong impact on economic outcome are scarce or vary widely among sources, need for further research into the value of that parameter may be indicated. We present two treatment scenarios, i.e., 3-d treatment and 8-d treatment, combined with the transmission scenario without contagious transmission ($R < 1$, specifically $R=0.21$). In each scenario, sensitivity analysis was performed for all input variables that are listed in Table 1.

RESULTS

The average economic benefit of treatment of chronic subclinical infection with *Str. uberis* or *Str. dysgalactiae* during lactation after 3-d or 8-d treatment on farms where the probability of transmission to other cows is low is shown in Table 1. The average net profit of 3-d treatment or 8-d treatment is €+11.62 and €–21.83, respectively. On farms where the probability of transmission is high, the average net profit of 3-d treatment or 8-d treatment is €68.60 and €58.62, respectively. All other scenarios yielded profits or losses in between these extremes.

Results of sensitivity analyses for the two treatment scenarios with low risk of transmission are shown in Table 2. The table lists the five input variables that had the strongest impact on economic return. This impact was based on the relative effect on economic return when compared with the relative change of the input variable. For example, if the input variable was changed by 10% and as a result, the economic return changed by more than 10%, the input variable was classified as being influential. Of all the input variables shown in Table 1, the influential input variables are (1) the probabilities of bacteriological cure and transmission (R), (2) the probability of culling together with the retention pay-off, and (3) the cost of antibiotics used for treatment. Changes in milk production losses did not have much impact on economic returns. The same variables were influential in sensitivity analysis of the treatment scenarios under high risk of transmission (results not shown).

Results for 3-d treatment in a herd where contagious transmission is unlikely indicate a net profit for most input variable values explored in the sensitivity analysis, provided that the bacteriological cure is at least 45–50% and the combined cost of diagnosis and treatment does not exceed €38. When contagious transmission is unlikely, 8-d treatment is not economically profitable, except for very valuable animals with a high retention pay-off, on farms where the

probability of culling due to subclinical mastitis exceeds approximately 20% or when the combined costs of diagnosis and antibiotic treatment remains below €50 (Table 2).

When contagious transmission of streptococci is likely, the net result of treatment would nearly always be positive according to our sensitivity analysis, irrespective of the level of other input variables. Note that this result is based on sensitivity analysis for one input variable at a time. If multiple input ariables are changed together, all in a direction of strong negative influence on economic profit, treatment may not be advantageous.

DISCUSSION

In Europe, where acceptable maximum levels for bulk milk SCC are much lower than in the USA (400,000 cells/ml v. 750,000 cells/ml) and where milk quotas are in place in many countries, antibiotics for treatment of subclinical mastitis during lactation are currently being marketed. The availability of these products, combined with results from recent research prompted us to re-examine the long-held view that treatment of *Str. agalactiae* mastitis during lactation is profitable (Yagamata et al. 1997), but that lactational treatment of subclinical mastitis caused by non-agalactiae streptococci is not economically justified (Craven, 1987; Wilson et al. 1999). We show that lactational treatment of chronic subclinical mastitis caused by *Str. dysgalactiae* or *Str. uberis* may also be economically beneficial.

Factors that we took into account and that have not been considered in previous economic calculations include the prevention of clinical flare-ups of subclinical infections (St. Rose et al. 2003) and the prevention of contagious transmission. Although non-agalactiae streptococci are often termed ‘‘environmental streptococci’’, *Str. dysgalactiae* is widely considered to be a contagious pathogen (Neave et al. 1969; Fox & Gay, 1993; Wang et al. 1999). *Str. uberis* does often have an environmental source (Wang et al.1999; Phuektes et al. 2001; Zadoks et al., 2003), but it may also spread from cow to cow (Phuektes et al. 2001; Zadoks et al. 2003). Measures that prevent contagious transmission reduce the prevalence of both *Str. dysgalactiae* and *Str. uberis* (Neave et al. 1969) and failure to treat infected animals or to use post-milking teat disinfection has been associated with outbreaks of *Str. uberis* mastitis (Cattell, 1996; Zadoks et al. 2001a, 2003). Strain typing is widely used in research to determine whether contagious transmission plays a role in a specific herd (Wang et al. 1999; Phuektes et al. 2001). As technology becomes cheaper, strain typing may become available for diagnostic purposes. Its use may improve insight in herd-specific epidemiology and assist in sound economic decision-making with respect to treatment of subclinical mastitis.

There is uncertainty and variability in many input parameters in our model. Sensitivity analysis indicated that the most important factors affecting the outcome of our economic analysis could be associated with the biology of mastitis and its causative agents, herd management, and economic factors such as retention payoff or cost of antibiotics. Some of these factors, e.g., the probability of cure, may be strain-dependent or cowdependent. For example, for *Staphylococcus aureus* it has been shown that some strains are more likely to cure than others (Sol et al. 2000), and also that some cows are more likely to cure than others, be it with (Sol et al. 1997, 2000)

or without treatment (Schukken et al. 1999). Some *Str. uberis* strains are more likely to cause chronic infections than others (Zadoks et al. 2003), but strain- or cow-specific factors that affect the probability of cure after treatment have not been determined. Further research on those topics is desirable for *Str. uberis* and *Str. dysgalactiae*.

Other factors, e.g., the probability of contagious transmission, may be strain dependent as well, as shown for Staph. aureus (Middleton et al. 2002) and *Str. uberis* (Zadoks et al. 2003), or management dependent, again as shown for Staph. aureus (Zadoks et al., 2002) and *Str. uberis* (Zadoks et al., 2001a). Because bacterial flora, cow characteristics and management differ widely between farms, the economic outcome of lactational treatment of chronic subclinical streptococcal mastitis may be highly farm-dependent.

Several of the factors that have an important impact on model outcome may differ between the two bacterial species we considered, *Str. dysgalactiae* and *Str. uberis*. On average, cure probabilities are higher for *Str. dysgalactiae* than for *Str. uberis* (DeLuyker et al. 2001) making a positive economic outcome more likely for treatment of *Str. dysgalactiae* mastitis. *Str. dysgalactiae* and *Str. uberis* also differ in contagiousness (Neave et al. 1969). Because contagiousness contributes to the profitability of economic treatment, as reflected in well-accepted feasibility of lactational treatment of the highly contagious *Str. agalactiae*, lactational treatment is again more likely to be profitable for *Str. dysgalactiae* than for *Str. uberis*. Data on *Str. dysgalactiae* are relatively scarce in the mastitis literature and its epidemiology is not entirely clear. The lack of information on *Str. uberis* and even more so for *Str. dysgalactiae* hampers an objective and detailed comparison of the two pathogens. As a generalization, epidemiology and response to treatment seem to favour treatment of *Str. dysgalactiae* over treatment of *Str. uberis* although management and strain differences between herds may make treatment economically (un) feasible for either species. Sensitivity analysis showed that 3-d treatment is profitable as long as combined costs of diagnosis and treatment does not exceed €38, and provided that at least 45–50% of treatments result in bacteriological cure. Thus, costs of culture (€3.95/sample, Animal Health Service, The Netherlands, data from 2003) and 3-d treatment (€27) would, on average, be offset by treatment revenues. Further analysis would be necessary to determine which specific diagnostic strategy (SCC-based or culture-based, at quarter, cow or herd level) would be economically most advantageous under various management conditions.

In contrast to 3-d treatment, 8-d treatment of chronic subclinical mastitis caused by *Str. dysgalactiae* or *Str. uberis* is, on average, not economically feasible, even if the prevention of clinical flare-ups and the cost that are prevented through prevention of new infections are considered. The higher costs of antibiotics and stronger reduction in profits due to milk withdrawal are not compensated for by the reduced costs due to a higher probability of bacteriological cure in comparison with 3-d treatment. Sensitivity analysis shows that 8-d treatment is profitable only for very valuable cows, when costs of diagnosis and treatment do not exceed €50 (which is well below the average retail price at the time this paper was written), or during an outbreak. Even then, it does not compare favourably with 3-d treatment. Treatment alone should never be considered the solution to an outbreak. Identification and removal of sources, be it infected animals or environmental sources, and management changes, must also be considered.

In this study, we assumed the costs of penalties due to high BTSCC, costs of other diseases resulting from mastitis, impact on fertility, and costs due to antibiotic residues in milk all to be zero. All these costs can be substantial on specific farms, but were considered to be of minor importance on an average farm and for the average cow. If any of these costs were included in the model, the economic benefit of lactational treatment of chronic subclinical streptococcal mastitis would increase. Another factor that has not been incorporated in our model but which must be considered is the risk of development of antimicrobial resistance when antimicrobials are used for treatment of subclinical streptococcal infections other than *Str. agalactiae*. Traditionally, antimicrobials were not used for this purpose, and the introduction of this usage may appear to lead to increased use of such products and hence an increased risk of contributing to the development of, or selection for, antimicrobial resistance. The main difference between our model, which predicts an economic benefit for the use of antimicrobials, and older models that did not predict such a benefit, is that we considered prevention of clinical flare-ups and transmission of infections to other animals. Both clinical flareups and infections in other animals, which could also be clinical, would be reasons for antimicrobial treatment under current management practices. If the increased use of antimicrobials for treatment of subclinical cases is offset by a decreased use of antimicrobials for new clinical cases, there may not be a net increase in use of antimicrobials. In fact, some models predict a decrease in mastitis prevalence and treatments in the long term when subclinical mastitis is treated with antibiotics (Zadoks et al. 2002). The hypothesis that lactational treatment of subclinical mastitis may improve udder health and reduce the net use of antibiotics in the long term is currently being tested in commercial dairy herds. Monitoring of antimicrobial resistance levels among streptococci is part of this study and should continue to be a concern when lactational treatment of clinical or subclinical mastitis is applied in dairy practice. Partial budgeting is a relatively simple method to assess economic profitability of the treatment of mastitis. It is particularly useful for relatively small changes on a farm, such as treatment v. no treatment of animals. However, as for any simple model, assumptions are relatively crude when compared with the complexity of reality. To address simplifications and assumptions like the ones used in our model and our sensitivity analysis, and to obtain more accurate estimates of the range of economic effects and the probability of specific outcomes within that range, a stochastic model would need to be developed to assess the profitability of treatment of subclinical mastitis due to non-agalactiae streptococci.

In conclusion, depending on circumstances such as prevailing bacterial flora, farm management and economic conditions, lactational treatment of chronic subclinical mastitis caused by *Str. dysgalactiae* or *Str. uberis* with antibiotics may or may not be economically beneficial. On farms where the probability of contagious transmission of causative agents is low, 3-d treatment is, on average, profitable but 8-d treatment is not (net profit +€11.62 and –€21.83 respectively). During outbreaks or in herds where contagious transmission is likely, both 3-d and 8-d treatments are profitable. In this situation, 3-d treatment is on average more profitable than 8-d treatment and 8-d treatment should probably only be considered for very valuable cows where the extra cost of antibiotics and discarded milk is offset by the higher probability of cure and the higher retention value of the cow. Identification of cow factors and bacterial strain characteristics associated with

cure and/or transmission would improve the cowand herd-specific estimates of the economic outcome of antibiotic treatment. Stochastic modelling will be needed to perform more accurate calculations of the range and probabilities of potential economic outcomes of lactational treatment of chronic subclinical *Str. dysgalactiae* and *Str. uberis* mastitis. Prudent use of antibiotics may favour treatment of subclinical infections when there is a net-reduction of antibiotic usage in the long term, but development of antimicrobial resistance should be monitored, whether treatment is used for subclinical mastitis, clinical mastitis, or both.

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CHAPTER 3

A partial budget model to estimate economic benefits of lactational treatment of subclinical *Staphylococcus aureus* mastitis

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ABSTRACT

Subclinical *Staphylococcus aureus* mastitis is rarely treated during lactation because it is widely believed to be uneconomical, although there are no economic studies that support this view. Partial budgeting was used to develop a deterministic simulation model to estimate the net cost or benefit of antibiotic treatment of subclinical *S. aureus* mastitis during lactation. Direct and indirect effects of treatment were taken into account, including prevention of clinical flare-ups and contagious transmission. Input variables were based on literature and on 2003–2004 prices in the Netherlands. When contagious transmission of *S. aureus* was likely (reproductive ratio R = 5.3), 3- and 8-d treatments resulted in an average net profit of €95.62 and €142.42, respectively, compared with no treatment. When the probability of *S. aureus* transmission was low (R = 0.32), the average economic benefit of 3- or 8-d treatment was −€21.12 and −€57.70, respectively. On low-transmission farms, 3-d treatment was profitable when the appropriate cows were selected for treatment using known risk factors for cure. Sensitivity analysis showed that the 6 most influential input variables in the model were chance of bacteriological cure, R, probability of culling, retention pay-off, and cost of antibiotics and bacterial culture. Although the economic outcome of lactational treatment of subclinical *S. aureus* mastitis is highly herd-, cow-, and strain-dependent, treatment is economically justified in many situations.

(**Key words:** *Staphylococcus aureus*, treatment, economic, partial budget)
Abbreviation key: BMSCC = bulk milk somatic cell count, **PC** = probability of cure, **PR** = penicillin-resistant, **PS** = penicillin-sensitive, **R** = reproductive ratio, **RPO** = retention pay-off.

INTRODUCTION

Staphylococcus aureus mastitis is a highly prevalent and costly disease. Control of *S. aureus* mastitis through preventive measures, dry cow treatment, and culling of infected animals can be economically profitable (Goodger and Ferguson, 1987; Zepeda et al., 1998). In addition to dry cow treatment, treatment of clinical mastitis is part of most standard mastitis control programs. By contrast, treatment of subclinical *S. aureus* mastitis during lactation is generally considered ineffective (Fox and Gay, 1993) and not economically justified when used as a stand-alone mastitis control strategy (Allore et al., 1998).

When treatment of *S. aureus* mastitis is postponed until the dry period, duration of infection increases. Indicators of chronic infection include prolonged periods or heightened levels of shedding of bacteria or somatic cells, palpable tissue changes, and infection of multiple quarters within a cow. All of the aforementioned factors are associated with a low probability of cure (Sol et al., 1997, 2000; Deluyker et al., 2005). In addition, long periods of infection and bacterial shedding create a prolonged window of opportunity for clinical mastitis to develop and for contagious transmission to occur (Lam et al., 1996; Zadoks et al., 2002a). Indirect effects of mastitis treatment (i.e., the prevention of clinical mastitis and transmission to other cows) are often overlooked in cost-benefit analyses but might tip the balance in favor of treatment (St. Rose et al., 2003; Swinkels et al., 2005). Some authors state that better cure rates are obtained by treating subclinical infections in lactation than by treating clinical cases of *S. aureus* mastitis (Bramley and Dodd, 1984). The chances of cure for cows that are subclinically infected with *S. aureus* can be improved by use of extended treatment (Deluyker et al., 2005) and by selection of treatmenteligible cows using current knowledge of risk factors for cure (Sol et al., 1997; Deluyker et al., 2005). Like inclusion of indirect effects, consideration of treatment and cow selection options will affect the outcome of costbenefit analysis for antimicrobial treatment.

To determine the profitability of lactational treatment with antimicrobials, potential benefits of treatment must be weighed against costs such as the price of antibiotics and loss of milk due to withholding times. The economic profitability of lactational treatment of subclinical mastitis has been demonstrated for *Streptococcus agalactiae* (Yamagata et al., 1987) and for nonagalactiae streptococci (Swinkels et al., 2005), but the value of lactational treatment of subclinical *S. aureus* mastitis has not been examined. We explored the economic benefits of treating subclinical *S. aureus* mastitis during lactation under European conditions [milk quota in place, legal limit for bulk milk somatic cell count (**BMSCC**) <400,000 cells/mL] using partial budgeting. In this analysis, effects at the cow level, such as bacteriological cure and prevention of clinical mastitis, and effects at the herd level, specifically reduced transmission potential, were taken into account. In addition, knowledge of risk factors for cure was incorporated in the economic model, resulting in cow-specific outcomes for the profitability of lactational treatment of subclinical *S. aureus* mastitis.

MATERIALS AND METHODS

Modeling Approach

Partial budgeting was used to develop a deterministic simulation model that estimates the net cost or benefit of treatment of subclinical *S. aureus* mastitis during lactation. Input variables were based on the literature and on dairy prices and conditions in the Netherlands from 2003 to 2004. The effect of 3-d treatment or 8-d treatment with antibiotics, both of which are registered for use in the Netherlands and other European countries, was compared with the effect of no treatment. In a partial budgeting model, economic effects are calculated as total revenues weighed against total costs. Treatment is profitable compared with no treatment when total revenues of treatment are higher than the total costs. Total revenues are calculated as extra revenue plus reduced costs. Total costs are calculated as reduced revenue plus extra costs. Costs and benefits of treatment were calculated at the cow level for one lactation.

First, a basic model was developed based on biological and economic parameters. Biological parameters included clinical outcome of infection with *S. aureus* and effects at herd level, specifically contagious transmission. The risk of transmission may vary considerably between herds, depending on herd management (Lam et al., 1996; Zadoks et al., 2002a) and strain factors (Smith et al., 1998; Zadoks et al., 2002a). Therefore, 4 scenarios were analyzed: 1) 3-d treatment in a herd with low risk of transmission, 2) 3-d treatment in a situation with high risk of transmission, 3) 8-d treatment with low risk of transmission, and 4) 8-d treatment with high risk of transmission. Economic parameters and calculations for each scenario are described. Numbers are rounded off in the text, but were not rounded off in calculations, which explains any discrepancies that may occur. Next, sensitivity analysis was performed for the 4 scenarios to identify input parameters with a strong impact on the model outcome. Finally, because cow and strain factors influence the probability of cure, the effect of these factors on economic benefits was explored.

Biological Parameters in Basic Model

Cure of persistent infections

In the Netherlands, the sampling interval for milk yield, SCC, and component testing is usually 4 wk. A cow is suspected of subclinical mastitis if at least 2 of 3 consecutive samples have cow-milk SCC above 250,000 cells/mL. It is recommended that a milk sample of such an animal be submitted for bacterial culture so that treatment and management decisions can be based on knowledge of the causative organism. If the causative organism is *S. aureus*, treatment can be considered. In this paper, we focus on treatment decisions for *S. aureus* infections that are detected using this sampling scheme, i.e., infections that have existed for at least 30 d (minimum of interval between 2 SCC measurements plus time needed for bacteriology). Such infections will be referred to as “persistent” throughout the paper.

Persistent subclinical mastitis may be left untreated, or a decision to treat can be made (Figure 1). Farmers can choose between short-term antibiotic treatment (3-d treatment) and extended

treatment (8-d treatment). Treatment can result in cure or in failure to cure. Untreated infections and infections that do not cure in response to treatment can cure spontaneously, continue to persist as subclinical infections, or develop into a clinical case of mastitis. Persistently infected animals may infect other animals. New infections may result in subclinical mastitis that cures spontaneously within 30 d, in persistent subclinical mastitis, or in clinical mastitis (Figure 1). All clinical cases are assumed treated and the model does not cover remission of clinical mastitis to subclinical mastitis.

The probability of spontaneous cure of persistent infections is low. Deluyker et al. (2005) describe a bestcase scenario of 6% cure for untreated *S. aureus* infections. This best-case scenario applies to heifers in late lactation with low levels of bacterial shedding. For older animals, animals at less than 200 DIM, and animals with higher shedding levels, the probability of cure (**PC**) is even lower than 6%. We assumed an average PC of 3% for untreated animals with persistent subclinical *S. aureus* infection (Figure 1). According to a meta-analysis of treatment trials of subclinical *S. aureus* mastitis, PC for the average cow is 35% after short-term antibiotic treatment (Sol et al., 1997). This average PC is similar to the midpoint of the range of probabilities described by Deluyker et al. (2005). For short-term treatment with pirlimycin, PC ranges from 3 to 56%. Probability of cure is 3% for a cow of parity >3 with high bacterial shedding levels in early lactation (worstcase scenario) and 56% for a heifer with low bacterial shedding levels in late lactation (best-case scenario). In the model, we used 35% as the chance of cure after 3-d treatment (Figure 1). Chances of cure increase with an increased duration of treatment (Ziv and Storper, 1985; Sol et al., 2000; Deluyker et al., 2005). After 8-d treatment, PC ranges from 10 to 86% (worst- and bestcase scenarios, respectively). For the model, we used the average PC for second- and third-lactation animals, which is approximately 60% (Deluyker et al., 2005; Figure 1).

According to a meta-analysis of treatment trials of subclinical *S. aureus* mastitis, PC for the average cow is 35% after short-term antibiotic treatment (Sol et al., 1997). This average PC is similar to the midpoint of the range of probabilities described by Deluyker et al. (2005). For short-term treatment with pirlimycin, PC ranges from 3 to 56%. Probability of cure is 3% for a cow of parity > 3 with high bacterial shedding levels in early lactation (worst-case scenario) and 56% for a heifer with low bacterial shedding levels in late lactation (best-case scenario). In the model, we used 35% as the chance of cure after 3-d treatment (Figure 1). Chances of cure increase with an increased duration of treatment (Ziv and Storper, 1985; Sol et al., 2000; Deluyker et al., 2005). After 8-d treatment, PC ranges from 10 to 86% (worst- and best- case scenarios, respectively). For the model, we used the average PC for second- and third-lactation animals, which is approximately 60% (Deluyker et al., 2005; Figure 1).

When infections persist, clinical flare-ups may occur. Lam (1996) reported 132 chronic *S. aureus* infections with subclinical onset. Twenty-three infections (17%) showed clinical flare-ups. Original data from Zadoks et al. (2002a) included 76 persistent *S. aureus* infections of which 16 (21%) showed clinical flare-ups. For the model, we used the average of the 2 percentages as the probability of clinical flare-ups, i.e., 19% of persistent infections were assumed to develop clinical signs (Figure 1).

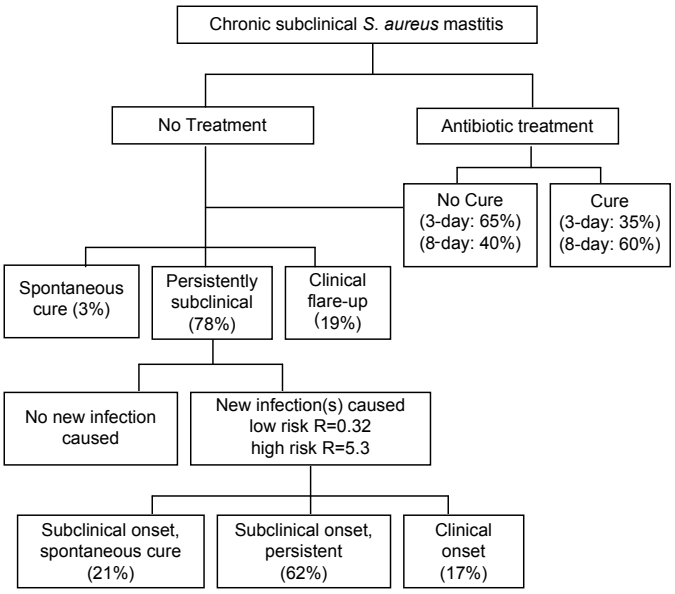

Figure 1. Deterministic model for effect of no treatment, 3-d treatment, or 8-d treatment on outcome of persistent subclinical *Staphylococcus* aureus mastitis. R = reproductive ratio (number of new infections caused by an existing infection).

Transmission of infections

Staphylococcus aureus is a contagious mastitis pathogen (Fox and Gay 1993; Lam et al., 1996). Subclinical *S. aureus* infections are a potential threat to uninfected cows and quarters in the herd. Contagiousness of a pathogen can be expressed in the transmission parameter, β, the average number of new infections caused by an infected individual per unit of time (Lam et al., 1996; Zadoks et al., 2002a). The actual number of new infections that is caused by an existing infection depends on the duration of the existing infection. The combined effect of contagiousness (β) and duration of infection (τ) is represented by the reproductive ratio R, which is the total number of new infections caused by an existing infection during its infectious lifetime. The reproductive ratio, R, can be presented as $\beta \times \tau$, or as β/α, where α is the cure rate and equals the inverse of the duration of infection, i.e., $\alpha = 1/\tau$.

The duration of *S. aureus* infection has been described by Lam et al. (1996, 1997) for 7 farms with low average annual BMSCC (<150,000 cells/mL). These data show that duration of infection differs with farm and management measures (postmilking teat disinfection vs. no disinfection). Average duration of infection was 115 d for disinfected quarters (Lam et al., 1997). For the model, the duration of infection for disinfected quarters was used, rather than the duration of infection for nondisinfected quarters, because postmilking teat disinfection is used on the majority of farms in the Netherlands (Barkema et al., 1998). Average duration of infection for herds with average annual BMSCC between 200,000 and 300,000 cells/mL also differed with herd and management strategy (Zadoks et al., 2002a). In a herd in which aggressive treatment or culling strategies were

not implemented, average duration of persistent *S. aureus* infections was 118 d (original data for herd A from Zadoks et al., 2002a), which is similar to results from low BMSCC herds.

The transmission parameter, β, has been estimated for disinfected and nondisinfected quarters in a steadystate situation and during an outbreak in low BMSCC herds (Lam et al., 1996), and for medium BMSCC herds under various management conditions (Zadoks et al., 2002a). As best-case scenario (indicated by the subscript bc), we used β_{bc} = 0.0028 infections/d for a steadystate situation with postmilking teat disinfection (Lam et al., 1996). From β_{bc} and τ, the reproduction ratio for the best-case scenario can be calculated as $R_{bc} = \beta_{wc} \times \tau$ = 0.0028 infections/d × 115 d = 0.32 infections (Figure 1). This value was slightly lower than values reported for medium BMSCC herds (0.40 to 0.75; Zadoks et al., 2002a). As worst-case scenario (indicated by the subscript wc), we used an outbreak situation in a herd where postmilking teat disinfection is not implemented. Estimates for β_{wc} and R_{wc} are 0.046 infections/d (Lam et al., 1996) and 0.046 infections/d × 115 d = 5.3 infections, respectively (Figure 1). As described above, a subclinical infection is considered persistent and eligible for treatment when it has existed for 30 d or more. Usually, there is some delay between detection of a cow that is suspected of infection and a treatment decision. Furthermore, the onset of infection may have preceded the first high SCC measurement as infections can occur on any day and SCC is measured periodically. For model calculations, we assumed the interval between onset of infection and onset of treatment to be 6 wk. Hence, if treatment is successful, it reduces duration of infection from 115 to 42 d, or by an average of 73 d compared with unsuccessful or no treatment. In a herd that implemented an aggressive strategy for detection and treatment of subclinically infected cows, observed duration for persistent subclinical infections was 51 d across cows, exemplifying that a lower average duration of infection can indeed be achieved under field conditions (original data for herd B from Zadoks et al., 2002a). In a low transmission scenario, a 73-d reduction in duration, equivalent to a prevented duration, τ_{prev}, of 73 d is associated with prevention of $\beta_{bc} \times \tau_{prev}$ = 0.0028 infections/d × 73 d = 0.20 new infections. In a high transmission scenario, the reduction in duration is associated with $\beta_{wc} \times \tau_{prev}$ = 0.046 infections/d × 73 d = 3.4 new infections. The probability of transmission of infections depends on herd management, but also on strain factors that are beyond the manager's control (Smith et al., 1998; Zadoks et al., 2002a). Therefore, a low or high transmission scenario cannot always be equated to good or poor udder health management.

When transmission occurs, the onset of a new infection can be clinical or subclinical. The proportion of new infections with clinical onset was 11.5% in herds with medium BMSCC (Zadoks et al., 2002a) and 22.8% on farms with low BMSCC (Lam et al., 1996). For the model, we used the arithmetic average, 17%, as probability that a new infection will have clinical onset (Figure 1). The remaining 83% of new infections have subclinical onset. Approximately 25% of new infections with subclinical onset or 21% of all new infections cure spontaneously within 30 d (original data from Zadoks et al., 2002a). The remaining infections, i.e., 100% − 17% − 21% = 62% become persistently subclinical (Figure 1).

Economic Parameters in Basic Model

Extra revenue

An increase in milk production after treatment would be considered extra revenue. There are no studies that describe milk production after treatment of persistent subclinical *S. aureus* infections. Mc-Dermott et al. (1983) found no increase in milk production after antibiotic treatment of high SCC cows compared with animals that did not receive treatment. Similarly, there was no increase in milk production after treatment of persistent subclinical mastitis caused by non-agalactiae streptococci (St. Rose et al., 2003). Based on those studies, we assumed milk production increase after treatment to be zero (Table 1).

Table 1. Overview of input variables used in the deterministic model and partial budget of economic benefit of lactational treatment of *Staphylococcus aureus* mastitis. Values for 8-day treatment are listed only when different from values for 3-day treatment.

Input Variable	Reference	Treatment	
		3-day	8-day
Extra revenue			
Increase in milk production after cure (kg)	St. Rose et al., 2003	0	
Reduced costs			
Prevention of clinical mastitis			
Costs of clinical flare-up of pre-existing subclinical mastitis (€)	De Vos and Dijkhuizen, 1998	187.0	
Reduction in probability of clinical mastitis after treatment (%)	Lam, 1996; Zadoks, 2002	6.0	10.8
Prevention of culling			
Culled animals (%)	NRS, 2003; this paper	12	
Retention pay off (€)	Based on Houben et al., 1994	506.0	
Reduction in probability of culling (%)	This paper	27	47
Prevention of new infections			
Cost of new clinical mastitis (€)	De Vos and Dijkhuizen, 1998	233	
Cost of lost milk production (€/kg)	De Vos and Dijkhuizen, 1998	0.07	
Cost of new short-duration subclinical mastitis (€)	This paper	1.98	
Costs of new persistent subclinical mastitis(€)	This paper	111.30	
Average costs of a new *S. aureus* infection (€)		109	
Reduction in probability of cure of a new infection (%)		34.0	58.2
Reduced revenue			
Value of discarded milk (€/kg)	De Vos and Dijkhuizen, 1998	0.14	
Duration of milk withhold (days)	This paper	6	11
Milk discard because of antibiotic residue (kg/day)	NRS, 2003	25.3	
Extra costs			
Antibiotics (€/day)	This paper	9	
Bacteriological culturing (€/test)	This paper	4.19	
Number of quarters tested	This paper	2	

Reduced costs

Successful treatment of persistent subclinical mastitis may prevent and thus reduce other costs. Costs can be reduced due to: 1) prevention of clinical mastitis in the infected cow; 2) prevention of culling of the infected cow; 3) prevention of new infections in other cows; 4) prevention of penalties for high SCC (Allore et al., 1998); and 5) prevention of losses due to poor fertility (Schrick et al., 2001). The last 2 factors were not considered in the model, although penalties and bonuses can be important factors in the economics of mastitis (Allore et al., 1998).

Reduced cost due to prevention of clinical mastitis

When a new *S. aureus* infection starts out as a clinical case of mastitis, its cost is estimated at €233 (De Vos and Dijkhuizen, 1998). This estimate includes the cost of lost milk production (estimated at €93), culling, antibiotic treatment, and discarded milk. Clinical *S. aureus* mastitis can also be the result of the flareup of a preexisting subclinical infection. In that case, some milk loss has already occurred during the subclinical phase of infection, and additional milk loss due to the clinical episode will be lower than for a clinical episode that signals the onset of a new infection. We assumed milk production loss associated with clinical flare-ups to be 50% less than for new infections. The resulting estimated cost for clinical flare-ups is €233 −(0.5 × €93) = €187 (Table 1). As described above, the probability of a clinical flare-up is estimated at 19% for persistent infections (Figure 1). After 3-d treatment, 65% of persistent infections remain uncured and 19% of those will show clinical flare-ups, so that 19% of 65% = 12.4% of quarters that receive 3-d treatment are expected to show clinical flare-ups. For 8-d treatment, 60% cure and 40% noncure is expected and 19% of 40% = 7.6% of quarters that receive 8-d treatment will develop clinical signs. Untreated quarters are expected to show clinical flare-ups in 19% of uncured infections, i.e., in 19% of 97% = 18.4% of untreated infections. Comparison of untreated and treated quarters shows that the reduction in probability of clinical flare-ups is 18.4% − 12.4% = 6.0% for 3-d treatment, and 18.4% − 7.6 % = 10.8% for 8-d treatment (Table 1). The associated reduction in cost is 6.0% or 10.8% of €187, i.e., €11.36 and €20.25, respectively (Table 2).

Reduced cost due to prevention of culling

Successful treatment decreases the probability of culling for infected animals. Of all culled animals, 5 to 17% are culled for mastitis-related reasons (Beaudeau et al., 1993; Esslemont and Kossaibati, 1997; Seegers et al., 1998; Whitaker et al., 2000). The high end of that range is close to the average culling percentage of 17.6% reported for a number of research herds in the Netherlands (Smolders, 1994). For our model, we assumed that 15% of all culls were due to mastitis. A large proportion of mastitis related culls, estimated at 50 to 65%, is due to subclinical mastitis (personal experience of and personal communications to J. M. Swinkels).

On Dutch farms, culling averages 34% of the herd annually (NRS, 2003–2004). Culling due to subclinical mastitis can now be calculated as the proportion subclinical out of proportion culled because of mastitis out of total proportion culled, i.e., 50% of 15% of 34% = 3% of the total number of cows in a herd. In a herd with average BMSCC of 200,000 to 300,000 cells/mL,

approximately 25% of animals are subclinically infected (Eberhart et al., 1982). Hence, the probability of culling for an individual subclinically infected cow is 3%:25% = 12% (Table 1). The retention pay-off (**RPO**) is an economic index that represents the future profitability of an animal. The RPO depends on parity, stage of lactation, pregnancy, milk production, and other factors. Calculations for RPO have been described by Houben et al. (1994). Using Houben's method, we calculated the RPO of a second-parity cow at 7 mo of lactation and 2 mo of gestation to be €506 (Table 1). Prices were adapted to the 2004 situation in the Netherlands.

Table 2. Net profit (€) of 3-day or 8-day lactational treatment of persistent subclinical *Staphylococcus aureus* mastitis compared to no treatment when risk of contagious transmission is low (R = 0.32) or high (R = 5.3). Net profit is calculated as (extra revenue + reduced costs) minus (reduced revenue + extra costs). R = reproductive ratio. Values for R = 5.3 are listed only when different from values for R = 0.32.

Input	**Net profit**			
Reproductive ratio	**0.32**		**5.3**	
Treatment	**3-day**	**8-day**	**3-day**	**8-day**
Extra revenue				
Milk production increase after cure	0	0		
Subtotal	0	0		
Reduced costs				
Revenues of reduced clinical mastitis	11.36	20.25		
Reduced costs due to culling	16.58	28.42		
Prevented costs new infections	7.57	12.97	124.30	213.09
Subtotal	35.51	61.64	152.25	261.76
Reduced revenue				
Withheld milk	21.25	38.96		
Subtotal	21.25	38.96		
Extra costs				
Antibiotics	27.00	72.00		
Bacteriological culture	8.38	8.38		
Labor	0	0		
Costs of penalty for antibiotic residue	0	0		
Subtotal	35.38	80.38		
Net result	**-21.12**	**-57.70**	**95.62**	**142.42**

Reduced costs due to culling can be calculated as the probability of culling without treatment minus the probability of culling after treatment multiplied by the costs of culling. Culling due to subclinical mastitis is largely limited to persistently infected animals. Without treatment, the probability of persistent subclinical infection is 78% (Figure 1). After 3-d treatment, the probability of persistent infection is 65% × 78% = 51%. Compared with untreated cows, this is a reduction in probability of persistent infection of 78% − 51% = 27% (Table 1). After 8-d treatment, the probability of persistent infection is 40% of 78% = 31% and the reduction in probability of persistent infection is 78% − 31% = 47% (Table 1). Twelve percent of persistently infected animals are assumed

culled, as described earlier. The average cost of culling due to subclinical mastitis is therefore 12% of the estimated cull cost of €506 per animal, i.e., €60.7. The reduction of costs due to culling is thus 27% of €60.7 = €16.58 and 47% of €60.7 = €28.42 for 3- and 8-d treatment, respectively (Table 2).

Reduced cost due to prevention of new infections

Although prevented cost of clinical mastitis and prevented cost of culling as described above pertain to the animal that receives treatment (i.e., the animal with the primary infection), there is also a reduced cost due to prevention of new or secondary infections in other animals. New infections can manifest as clinical mastitis, subclinical mastitis with short duration, or persistent subclinical mastitis (Figure 1), and each manifestation is associated with a cost. The reduced cost due to prevention of new infections is based on the average cost of a new infection, and the number of new infections that is prevented by a specific treatment given the level of risk of contagious transmission. As quoted previously, the cost of a new infection that manifests as clinical mastitis is estimated at €233 (De Vos and Dijkhuizen, 1998) (Table 1). The cost of a new case of subclinical mastitis is primarily the result of reduced production and, if the subclinical mastitis becomes persistent, of potential clinical flare-ups and culling. To avoid a snowball effect and keep the model manageable, we did not include the cost of tertiary infections that are potentially prevented by prevention of secondary infections.

Milk production losses resulting from subclinical mastitis are correlated with SCC. Production loss for a cow with SCC of 50,000 cells/mL or below is set at zero (Hortet and Seegers, 1998). Every doubling of SCC is associated with a production loss of 0.4 kg/d for heifers and 0.6 kg/d for cows (Hortet and Seegers, 1998). The average SCC of quarters with persistent subclinical *S. aureus* infection is $e^{7.13}$ = 1.2 × 106 cells/mL (Table 1 from Deluyker et al., 2005). In the Netherlands, the average number of infected quarters per *S. aureus*-infected cow is 1.675 (Poelarends et al., 2001). When the remaining 2.375 quarters of a cow are assumed uninfected, the average SCC of a cow with persistent subclinical *S. aureus* mastitis is estimated at [1.675 × 1.2 × 106 + 2.375 × 50,000]/4 = 5.5 × 105 cells/mL. This is a 3.5-fold doubling of the healthy baselineSCC of 50,000 cells/mL, and is thus associated with a production loss of 3.5 × 0.4 = 1.4 kg/d for heifers and 3.5 × 0.6 = 2.1 kg for cows. If there is no milk quota system (as in the United States), the cost of lost milk production is equal to the amount of lost milk multiplied by the milk price. In the European Union, where a quota system is in place, loss of milk is usually compensated for by the extra milk production of animals that are kept in the herd beyond their intended cull date. Therefore, the cost of lost milk production is lower when a milk quota is in place than in a nonquota situation; this cost is estimated at €0.07/kg of milk (De Vos and Dijkhuizen, 1998; Table 1).

A new subclinical infection can cure spontaneously within 30 d, i.e., before the next routine milk sampling. We assumed the average duration of a spontaneously cured infection to be 15 d. For a heifer, the production loss associated with short-term subclinical infection was calculated to be 1.4 kg/d × 15 d = 21 kg. For a multiparous cow, it was calculated as 2.1 kg/d × 15 d = 32 kg. Assuming that approximately one-third of a herd consists of heifers, the average costs of new infections that cure spontaneously within 30 d are estimated to be (1/3 × 21 + 2/3 × 32) kg

× €0.07/kg = €1.98 (Table 1). For new subclinical infections that persist, average duration was estimated at 115 d, and the average production loss was estimated at 1.4 kg/d × 115 d = 161 kg for heifers, and 2.1 kg/d × 115 d = 242 kg for multiparous cows. The average estimated milk production loss would be 1/3 × 161 kg + 2/3 × 242 kg = 215 kg. This amounts to a loss of 215 kg × €0.07/kg = €15.05. Persistent subclinical mastitis also has the cost associated with clinical flare-ups and culling. The cost of flare-ups is €187 and this cost is incurred in 19% of persistent infections, amounting to an average loss of 19% × €187 = €35.53 per persistent infection. The cost associated with culling is €60.72, as calculated for treated cows. Thus, the total cost of new subclinical infections that become persistent is €15.05 + €35.53 + €60.72 = €111.30 (Table 1).

New infections that result from transmission can have clinical onset (17% of infections, cost €233 per infection), subclinical onset and short duration (21% of infections, cost €1.98 per infection), or subclinical onset and long duration (62% of infections, cost €111.30 per infection), resulting in an average cost of new infections of 0.17 × €233 + 0.21 × €1.98 + 0.62 × €111.30 = €109 (Table 1). Probability of cure after treatment is 35 and 60% for 3- and 8-d treatment, respectively. The remaining 65 and 40% of animals have a 3% chance of curing spontaneously, as do untreated animals. Total PC is thus 3% for untreated animals, 35% plus 3% of 65% = 37% for animals after 3-d treatment, and 60% plus 3% of 40% = 61.2% after 8-d treatment. Hence, 3-d and 8-d treatments are associated with a 34% and a 58.2% higher chance of cure, respectively, than no treatment (Table 1). As described under “transmission of infections,” the number of prevented infections that can be attributed to an infection that is cured is estimated at 0.2 in a low-transmission scenario, and at 3.4 in a high-transmission scenario. The prevented costs due to prevention of transmission of infection can now be calculated as 34% of 0.2 × €109 = €7.57 for 3-d treatment in a low-transmission herd, 34% of 3.4 × €109 = €124.30 for 3-d treatment in a high-transmission herd, 58% of 0.2 × €109 = €12.97 for 8-d treatment in a lowtransmission herd, and 58% of 3.4 × €109 = €213.08 for 8-d treatment in a high-transmission herd (Table 2).

Reduced revenue

Treatment with antibiotics results in residues in the milk; this milk is not suitable for human consumption and has to be discarded. Withholding or discarding milk leads to reduced revenue. The average costs of discarded milk were estimated at €0.14/kg. (De Vos and Dijkhuizen, 1998; Table 1). When a quota system is in place, the cost of discarded milk is higher than the cost of milk that is lost due to high SCC. The difference in economic value arises because the cost of concentrates is included in the value for discarded milk but not in the value of nonproduced milk. We assumed the milk-withholding period after completion of treatment to be 3 d. This is based on the average withholding period of 4 d of the 2 currently registered and marketed antibiotics on the Dutch market and the assumption that the day of last treatment is counted as a day for withholding milk. Thus, total milk withhold times are 6 and 11 d for 3-d and 8-d treatment, respectively. Average milk production was assumed 25.3 kg/d (NRS, 2003–2004; Table 1). Total costs of withheld milk can be calculated as 6 d × 25.3 kg/d × €0.14/kg = €21.25 and 11 d × 25.3 kg/d × €0.14/kg = €38.96 for 3- and 8-d treatment, respectively (Table 2).

Extra costs

The extra costs of treatment vs. no treatment are costs of antibiotics, diagnostic testing, extra labor, and potential penalties due to antibiotic residues in milk. The cost of antibiotics depends on the choice of drug, route of administration, dosage, and length of treatment. Registered products for the treatment of subclinical mastitis in the Netherlands are commercially available to producers at an average cost of €9/d (Table 1). This results in extra costs of €27 and €72 for 3- and 8-d treatments, respectively (Table 2). Risk factors for cure of persistent subclinical mastitis include the location of the infected quarter(s) and penicillin sensitivity (**PS**) of the *S. aureus* strain that causes infection (Sol et al., 1997, 2000; Deluyker et al., 2005). When treatment decisions are to be made using knowledge of these risk factors, quarter location and PS need to be determined. Quarter location can be determined using the California mastitis test. The cost of the California mastitis test was considered negligible. To determine PS, bacteriological culture and PS testing are needed. The cost of such testing is €4.19 per quarter (Animal Health Service, Deventer, the Netherlands; Table 1). Taking into consideration that cows infected with *S. aureus* have more than one infected quarter on average (Poelarends et al., 2001), we used an average cost of testing of 2 × €4.19 = €8.38 per cow (Table 2). In some cases, testing will not result in detection of *S. aureus*, and *S. aureus* treatment will not be given. This decreases the extra cost of treated cows relative to nontreated cows. The probability of negative cultures is highly farm-dependent. Culture may also result in detection of *Streptococcus dysgalactiae* or *Streptococcus uberis*, which could be a reason for treatment (Swinkels et al., 2005). Because it is difficult to estimate the cost of culture for *S. aureus* negative cows and because a decision not to treat can be made without culture, whereas a decision to treat is assumed culture-based, we did not include costs of negative cultures in the model. Extra costs for labor have to be taken into account if time saved by not treating the animal can be used for other paid labor. This is not likely on the majority of farms in the Netherlands because most dairy farms are family farms where farm income is based on the amount of milk that is produced, not on hours of labor. Therefore, we assumed the extra costs of labor to be zero (Table 2). Extra costs resulting from penalties for antibiotic residue in the bulk tank are assumed zero because these costs can and should be prevented through good management (Table 2).

Sensitivity Analysis for Basic Model

Sensitivity analysis is used to calculate what happens to model output when one input variable at a time is changed from the average situation. Sensitivity analysis was performed for 4 scenarios, i.e., 3-d treatment or 8-d treatment combined with low (R = 0.32) or high (R = 5.3) risk of *S. aureus* transmission. For each scenario, the impact of a change in model input on model output was calculated. The impact of all input variables listed in Figure 1 or Table 1 on model output was determined. For the 6 most influential input variables, the impact of multiple levels of the variable on model output was tabulated (Table 3).

Table 3. Sensitivity analysis: Effect of the six most influential input variables for 3-day or 8-day treatment of subclinical *Staphylococcus aureus* mastitis on net profit in herds with low (R = 0.32) or high risk of transmission (R = 5.3).

Input	**Net profit**			
Reproductive ratio	**R = 0.32**		**R = 5.3**	
Treatment	**3-day**	**8-day**	**3-day**	**8-day**
Bacteriological cure (%)				
10	-47.24	-109.95	-13.89	-76.60
20	-36.79	-99.50	29.91	-32.80
35 (This paper 3-d treatment)	-21.12	-83.83	95.62	32.91
50	-5.44	-68.15	161.32	98.61
60 (This paper 8-d treatment)	5.01	-57.70	205.13	142.42
80	25.91	-36.80	292.73	230.02
90	36.36	-26.35	336.54	273.83
100	46.81	-15.90	380.34	317.63
Proportion (%) culled for subclinical mastitis				
0	-40.31	-90.60	36.12	40.42
12 (This paper)	-21.12	-57.70	95.62	142.42
20	-8.33	-35.77	135.28	210.41
Retention pay off (€)				
0	-40.31	-90.60	36.12	40.42
506 (This paper)	-21.12	-57.70	95.62	142.42
1000	-2.38	-25.59	153.70	241.99
Antibiotic costs (€)				
15	-9.12	-0.70	107.62	199.42
27 (This paper; 3-d treatment)	-21.12	-12.70	95.62	187.42
50	-44.12	-35.70	72.62	164.42
72 (This paper. 8-d treatment)	-66.12	-57.70	50.62	142.42
Price of discarded milk (€)				
0.00	0.13	-18.74	116.87	181.38
0.07	-10.50	-38.22	106.24	161.90
0.14 (This paper)	-21.12	-57.70	95.62	142.42
0.21	-31.75	-77.18	84.99	122.94
0.30	-45.41	-102.23	71.33	97.89
Costs of bacterial culture (€)				
0.00	-12.74	-49.32	104.00	150.80
4.19 (1 sample)	-16.93	-53.51	99.81	146.61
8.38 (2 samples. This paper)	-21.12	-57.70	95.62	142.42
16.76 (4 samples)	-29.50	-66.08	87.24	134.04

Impact of Risk Factors for Cure

In the basic model, an average probability of bacteriological cure of 35 and 60% was used for 3-d and 8-d treatments, respectively. However, bacteriological cure of *S. aureus* infections is not a random event. Host, pathogen, and management factors that influence PC include parity, stage of lactation, quarter location, quarter SCC, PS, and duration of treatment (Sol et al., 1997, 2000; Deluyker et al., 2005). The impact of various variables on cure has been quantified, which enables us to predict PC for each cow-pathogen-treatment combination. The prediction equation was adapted from Sol and colleagues (1997) and is described by: PC = 100 × 1/{1 + EXP[−1 − (intercept + parity + stage of lactation + quarter location + quarter SCC + PS + treatment regimen)]}. This prediction equation differs from that originally given for lactational treatment of subclinical mastitis in that the effects of PS and treatment regimen were added to the equation. Probability of cure after treatment of subclinical *S. aureus* mastitis was not significantly different between PS and penicillin-resistant (**PR**) strains in the original analysis, but a considerable numerical difference was observed, i.e., 35% cure for PS strains and 25% cure for PR strains (Sol et al., 1997). In a similar study of risk factors for cure after treatment of clinical *S. aureus* mastitis, PS strains were again associated with a higher probability of cure than were PR strains (58% for PS vs. 41% for PR), and this difference was significant (Sol et al., 2000). Ziv and Storper (1985) report 56 and 32% cure after treatment of clinical *S. aureus* mastitis caused by PS and PR strains, respectively. Because PS was repeatedly shown to influence the chance of cure and because PS is determined for *S. aureus* isolates by the Dutch Animal Health Service as part of routine bacteriological testing, we decided to include this variable in the model. Similarly, although the original study of lactational treatment of subclinical *S. aureus* mastitis (Sol et al., 1997) did not consider treatment duration, a study of treatment of clinical mastitis by the same authors (Sol et al., 2000), a study in 8 dairy herds (Ziv and Storper, 1985), and a large multicenter study comparing short and extended lactational therapy of subclinical mastitis (Deluyker et al., 2005) showed a significant effect of treatment duration on cure.

In the prediction equation, intercept (0.40) and regression coefficients from Sol et al. (1997) were used for parity (−1.05 for parity >2), DIM (−1.90 for DIM ≤ 100, −0.95 for 100 < DIM ≤ 200), quarter position (−1.53 for hind quarter), and quarter SCC (−1.25 for lnSCC >6.9). The regression coefficient for PS vs. PR was calculated from the average cure rate for PS and PR infections (35 and 25%, respectively) and was −0.49 for infections with PR *S. aureus*. The regression coefficient for extended treatment was calculated using best-case scenarios for short and extended treatment. According to Sol et al. (1997), the best-case PC is 61%, which is very similar to the best-case PC of 56% reported by Deluyker et al. (2005) for short-term treatment. In both studies, the best-case scenario applies to a first-lactation animal at more than 200 DIM. If such an animal is treated for 8 d, PC is 86% (Deluyker et al., 2005). Using the prediction equation and 61% vs. 86% as PC, the regression coefficient for extended therapy was calculated at +1.42. Our best-case scenario then becomes a heifer at >200 DIM that is infected in a front quarter with a PS *S. aureus* strain resulting in lnSCC <6.9 and that receives extended therapy. Its PC = 100 × 1/{1 + EXP[−1 × (0.40 + 0 + 0 + 0 + 0 + 0 + 1.42)]} = 86%. The worstcase scenario is an older animal at <100 DIM infected in a hind quarter with a PR strain resulting in lnSCC >6.9. Short-duration treatment of this animal would result in PC = 100 × 1/{1 + EXP[−1 × (0.40 − 1.05 − 1.90 − 1.53 − 1.25 − 0.49 + 0)]} = 0.3%.

RESULTS

Net profits for the basic model are presented in Table 2 for 3-d and 8-d treatments under low- and high-transmission conditions. In herds where contagious transmission of *S. aureus* is very likely (R = 5.3), 3-d and 8-d treatment result in high positive net profits of €95.62 and €142.42, respectively. When the probability of *S. aureus* transmission is low (R = 0.32), the average economic benefit of both treatments is negative with a calculated net profit of −€21.12 and −€57.70 for 3-d and 8-d treatment, respectively.

Bacteriological cure, culling due to subclinical mastitis, retention pay-off, antibiotic costs, price of discarded milk, and bacteriological culturing were the 6 most influential input variables identified during sensitivity analysis of the basic model. Table 3 shows the net benefit at various levels of each variable for the 4 scenarios covered in this paper. For example, in herds with low transmission (R = 0.32), 3-d treatment was profitable if the chance of cure was higher than approximately 55%. In herds with high transmission (R = 5.3), 3-d and 8-d treatments were profitable at predicted cure probabilities of 13 and 28%, respectively.

A detailed analysis of the effect of host- and pathogenspecific risk factors on net benefit is presented in Tables 4 and 5. To limit the size of the tables, they do not include all possible combinations of parity, SCC, DIM, and quarter. Combinations with a predicted net profit less than −€51 for 3-d treatment (Table 4) or less than −106 for 8-d treatment (Table 5) are not shown. Probability of cure ranges from less than 1 to 86% and associated net benefits range from less than −€106 to +€257 (Table 5). In herds with low transmission of *S. aureus*, 3-d treatment is only expected to be profitable for young animals in late lactation that are infected in front quarters. In herds with high transmission of *S. aureus*, 3-d treatment can be profitable for both young and old animals. However, older animals would have to be infected in front quarters, have low cow-milk SCC, and be treated in late lactation for treatment to be economically justified (Table 4). Extended treatment is not economically profitable in herds with low transmission of *S. aureus*. By contrast, if the risk of transmission is high, 8-d treatment is economically justified for the majority of risk factor combinations (Table 5). The bestand worst-case transmission scenarios shown in Tables 4 and 5 are extremes and occur in a minority of herds only. In many herds, *S. aureus* prevalence is more or less constant, implying that every infection is on average replaced by one new infection, i.e., R = 1. Net benefits for herds in which R = 1 were slightly higher than to those for low-transmission herds. At R = 1, a positive net result was obtained for 3-d treatment of young animals with low cow-milk SCC and infected front quarters in late lactation, irrespective of antibiotic sensitivity of the organism, and for 8-d treatment of such animals with infection by a PS strain (results not shown). Note that Tables 4 and 5 include cow-specific risk factors for cure, but not other cow-specific factors that may affect net benefit, such as RPO or milk yield.

DISCUSSION

It is widely believed that treatment of subclinical *S. aureus* mastitis during lactation is ineffective and uneconomic. Nevertheless, antibiotics that are specifically registered for lactational treatment of

subclinical mastitis are being marketed and promoted in Europe, where the maximum acceptable level of BMSCC is 400,000 cells/mL. Treatment of persistent subclinical streptococcal mastitis during lactation can be profitable when indirect effects of cure (i.e., prevention of clinical flare-ups and prevention of transmission to other animals) are taken into account (Swinkels et al., 2005). In the cost-benefit analysis presented here, we show that the profitability of lactational treatment of *S. aureus* mastitis depends strongly on host, pathogen, and herd factors and that treatment is economically justified in a variety of situations.

Treatment is economically profitable for many cows when the likelihood of contagious transmission of *S. aureus* is high. Although some strains of *S. aureus* can be highly contagious even under good management conditions (Smith et al., 1998), contagious transmission is usually the result of suboptimal udder health management, e.g., failure to use postmilking teat disinfection (Lam et al., 1996; Barkema et al., 1998). When udder health management is suboptimal, management changes need to be implemented to reduce the incidence of infection. In the Netherlands, close to 40% of farmers do not implement postmilking teat disinfection (Barkema et al., 1998); use of teat dips with proven efficacy should be recommended on such farms. Successful treatment of persistent *S. aureus* mastitis with antibiotics will also result in reduced incidence of new infections because it decreases the exposure of uninfected individuals to the pathogen. The herd level effect of treatment is an important element of its economic justification, as shown by the difference between low- and high-transmission scenarios in Tables 2, 3, 4, and 5. It must be emphasized that the term “persistent” *S. aureus* mastitis is used in our paper to describe any infection that has been present for 30 d or more. That does not imply that all cows that have been infected for long periods should be considered for treatment.

On the contrary, cows with induration of udder tissue, multiple infected quarters, or other indicators of very persistent infection should not be selected for treatment. Some treatment protocols explicitly exclude cows with palpable udder changes from antibiotic treatment (Deluyker et al., 2005). Thus, when a *S. aureus* control program is initiated, it may be necessary to implement a 3-pronged approach: 1) improve herd management, specifically milking routines; 2) identify cows that are not eligible for treatment and cull or segregate them (Wilson et al., 1995) or use early dry-off; and 3) treat cows that have a considerable chance of cure. Risk factors that predict the success of treatment have been identified in a number of countries and studies and are largely consistent across treatment and study protocols (Ziv and Storper, 1985; Sol et al., 1997, 2000; Deluyker et al., 2005). When the right cows, the right infections, and the right treatment duration are selected, the chance of cure after lactational treatment of persistent subclinical *S. aureus* mastitis can exceed 50%. The sooner an infection is detected and treated, the higher the chance of cure. When a control program is first implemented, some existing infections will already be intractable, whereas extended treatment may be needed to cure others. Once the control program is in place, early detection and treatment of new infections is feasible and short-term treatment may be preferable from an economic perspective.

In herds with low risk of *S. aureus* transmission, treatment of an average cow with persistent subclinical *S. aureus* mastitis is not profitable. Three-day treatment can be profitable in such herds when cows are selected with an expected cure probability of approximately 55% or higher (Table 4).

Table 4. Sensitivity analysis of host, pathogen and treatment factors on net benefit of 3-day treatment of subclinical *Staphylococcus aureus* mastitis during lactation on farms with low (R = 0.32) or high risk of transmission (R = 5.3). R = reproductive ratio. Positive net benefits are bolded.

Pathogen	PC[1] (%)	Cow Factors				R = 0.32	R = 5.3
		Parity[2]	SCC[3]	DIM[4]	Quarter		
PS[5]	18.2	young	low	early	front	-39	**22**
	36.6	young	low	mid	front	-19	**103**
	59.9	young	low	late	front	**5**	**205**
	6.0	young	high	early	front	-51	-31
	14.2	young	high	mid	front	-43	**5**
	29.9	young	high	late	front	-26	**73**
	11.1	young	low	mid	hind	-46	-10
	24.4	young	low	late	hind	-32	**49**
	8.5	young	high	late	hind	-49	-20
	7.2	old	low	early	front	-50	-26
	16.8	old	low	mid	front	-40	**16**
	34.3	old	low	late	front	-22	**93**
	5.5	old	high	mid	front	-52	-34
	13.0	old	high	late	front	-44	-1
	10.2	old	low	late	hind	-47	-13
PR[5]	12.0	young	low	early	front	-45	-5
	26.1	young	low	mid	front	-30	**57**
	47.8	young	low	late	front	-8	**152**
	3.8	young	high	early	front	-54	-41
	9.2	young	high	mid	front	-48	-17
	20.8	young	high	late	front	-36	**33**
	2.9	young	low	early	hind	-55	-45
	7.1	young	low	mid	hind	-50	-27
	16.5	young	low	late	hind	-40	**15**
	5.4	young	high	late	hind	-52	-34
	4.6	old	low	early	front	-53	-38
	11.0	old	low	mid	front	-46	-10
	24.2	old	low	late	front	-32	**48**
	3.4	old	high	mid	front	-54	-43
	8.4	old	high	late	front	-49	-21
	2.6	old	low	mid	hind	-55	-46
	6.5	old	low	late	hind	-51	-29

1) PC = Predicted cure
2) Parity: young - 1st or 2nd parity; old - 3rd or higher parity
3) SCC: low - lnSCC < 6.9; high - lnSCC 6.9 or higher
4) LS = Lactation Stage: early <100 DIM; mid 100<DIM≤ 200; late >200 DIM
5) PS =Penicillin Sensitive, PR = Penicillin Resistant

Table 5. Sensitivity analysis of host, pathogen and treatment factors on net benefit of 8-day treatment of subclinical *Staphylococcus aureus* mastitis during lactation on farms with low (R = 0.32) or high risk of transmission (R = 5.3). R = reproductive ratio. Positive net benefits are bolded.

Pathogen	PC[1] (%)	Cow risk factors				R = 0.32	R = 5.3
		Parity[2]	SCC[3]	DIM[4]	Quarter		
PS[5]	48.0	young	low	early	front	-70	**90**
	70.5	young	low	mid	front	-47	**188**
	86.1	young	low	late	front	-30	**257**
	20.9	young	high	early	front	-99	-29
	40.6	young	high	mid	front	-78	**57**
	63.9	young	high	late	front	-54	**159**
	16.7	young	low	early	hind	-102	-47
	34.1	young	low	mid	hind	-85	**29**
	57.2	young	low	late	hind	-61	**130**
	27.7	young	high	late	hind	-91	**1**
	24.4	old	low	early	front	-95	-13
	45.5	old	low	mid	front	-73	**79**
	68.4	old	low	late	front	-49	**179**
	19.3	old	high	mid	front	-100	-36
	38.2	old	high	late	front	-80	**47**
	31.9	old	low	late	hind	-87	**19**
PR[5]	36.1	young	low	early	front	-83	**38**
	59.4	young	low	mid	front	-58	**139**
	79.1	young	low	late	front	-37	**226**
	13.9	young	high	early	front	-106	-59
	29.5	young	high	mid	front	-89	**9**
	52.0	young	high	late	front	-66	**107**
	24.0	young	low	mid	hind	-95	-15
	45.0	young	low	late	hind	-73	**77**
	19.0	young	high	late	hind	-100	-35
	16.5	old	low	early	front	-103	-48
	33.8	old	low	mid	front	-85	**27**
	57.0	old	low	late	front	-61	**129**
	27.5	old	high	late	front	-92	**0**
	22.3	old	low	late	hind	-97	-23

1) PC = Predicted cure
2) Parity: young - 1st or 2nd parity; old - 3rd or higher parity
3) SCC: low - lnSCC < 6.9; high - lnSCC 6.9 or higher
4) LS = Lactation Stage: early <100 DIM; mid 100<DIM≤ 200; late >200 DIM
5) PS =Penicillin Sensitive, PR = Penicillin Resistant

For example, first- or second-parity cows infected in a front quarter with a PS strain, with cow-milk SCC <1 million cells/mL, and >200 DIM are eligible for treatment. Extended treatment is not profitable in low-transmission herds. Longer treatment will lead to a higher chance of cure but this benefit does not outweigh the higher costs of antibiotics and discarded milk. The interpretation of the economic benefit of treatment in low transmission herds is complicated by the fact that routine testing, potentially followed by lactational treatment of infected animals, is one of the factors that contributes to low risks of transmission (Smith et al., 1998; Zadoks et al., 2002a). In other words, the success of treatment leads to a reduction in exposure and contributes to a lowtransmission scenario. In the low-transmission situation, the economic benefit of treatment is subsequently limited. Routine testing of milk samples for SCC or mastitis pathogens also contributes to reduced transmission through increased awareness of the importance of udder health management (Hillerton et al., 1995; Zadoks et al., 2002a).

The biological and economic models presented in this paper have limitations, as do all models. Partial budgeting is a relatively simple method to calculate economic effects. It is useful in studies that compare relatively small changes in a system such as implementation of a test and cull (Goodger and Ferguson, 1987) or treatment program (Swinkels et al., 2005) vs. no implementation of a control program. The input variables of a partial budget model are fixed averages that are crude descriptors of reality. For example, the model does not take into consideration that there may be herdlevel constraints that limit the feasibility of treatment of culling of infected cows. In addition, the deterministic partial budget model allows culling or treatment of fractional cows or quarters. This problem can be addressed by use of a stochastic model, in which a whole cow or quarter is culled or treated and a random number generator is used to make this decision. A stochastic model also allows for estimation of the range of possible model outcomes, and for quantification of the likelihood of each outcome (Allore et al., 1998). Some events are unlikely to happen, but may have a very severe influence if they do happen, such as the outbreak of mastitis that resulted from the introduction of a novel *S. aureus* strain in the Washington State University herd (Smith et al., 1998). That outbreak shows that in addition to economic considerations, risk analysis may need to be part of herd management, including weighing of the likelihood of events, and the magnitude of potential consequences. A stochastic model can support riskbased decision-making. In addition to stochasticity or variability in input variables, there is uncertainty for many input variables in our model. The most important input variables in this study were bacteriological cure, probability of transmission, the duration of infection, the chance of culling due to subclinical mastitis, RPO, antibiotic costs, price of discarded milk, and costs of bacteriological culture (Table 3). When applying the economic model for on-farm treatment decisions, herdspecific values should be used for these economically important input variables whenever possible. If data on important input variables are scarce, further research on such topics is indicated. For example, R is an important input variable in the economic model, but estimates of R are scarce due the costly nature of studies needed to determine R or its components (Lam 1996; Zadoks et al., 2002a). A very crude estimate of R can be obtained under field conditions based on the change in number of cows with elevated SCC. When R = 1, each infected individual replaces itself on average with one new infected individual and the total number of infected individuals would not change. Thus,

if the number of cows with elevated SCC increases, the crude estimate for R is greater than 1. If the number of cows with elevated SCC decreases, the crude estimate for R is less than 1. The number of infected cows at any point also depends on treatment and culling decisions and on the number of infections of environmental origin (Zadoks et al., 2002a).

The current model does not address potential interdependence of input variables. Interdependence of variables may exist at the herd level, the cow level, or the pathogen level. Cow level factors that affect multiple input parameters of the model include parity, milk production, and duration of infection. Younger animals have a higher probability of cure (Tables 4 and 5) as well as a higher RPO than older animals, due to a longer productive life expectancy. Table 3 shows that an increase in RPO is associated with higher net profit of lactational *S. aureus* treatment. Thus, both PC and RPO favor treatment of young animals. The influence of milk production on economic benefit of treatment is more complex. High milk production increases the RPO of an animal (Houben et al., 1994) but high milk production is also associated with high losses due to discarded milk. Thus, high milk production contributes and detracts from the economic benefit of treatment. For an animal with high production potential (high RPO), treatment may only be cost-effective in late lactation when the actual yield and loss due to discarded milk are low, and the expected benefits are high due to the higher PC associated with DIM > 200. Early treatment, which requires early detection, is beneficial at the herd level and cow level. Compared with late detection of infection, detection of infection in its early stages is associated with lower average SCC (higher chance of cure), lower number of infected quarters (lower cost of culture and sensitivity testing; fewer quarters that need to cure), and longer prevented duration of infection (more prevented costs). All factors favor treatment of infections that are detected early after onset. For cows that are in early lactation, these benefits may outweigh the disadvantage that early-lactation animals have compared with late-lactation animals in terms of PC. In other words, the economic loss associated with treatment in early lactation may be smaller than the economic loss associated with postponement of such treatment until late lactation. Currently, little is known about correlations among strain characteristics that may affect the net benefit of treatment. Several studies have shown that *S. aureus* strains may differ in their ability to cause intramammary infections (Zadoks et al., 2002b), clinical mastitis (Zadoks et al., 2000), or mastitis outbreaks (Smith et al., 1998). The majority of *S. aureus* infections on multiple continents are caused by a limited number of cow- and udder-adapted strains, as recently shown by multilocus sequence typing (Smith et al., 2005). It is conceivable that udderadapted strains show a higher probability of transmission and a lower chance of cure than strains that are not adapted to this specific niche. In that case, a strain might be associated with low PC, which would not favor treatment, and high R, which, by contrast, would increase the economic benefit of treatment. Despite its limitations, this model is the best available estimate of the net cost or benefit of lactational treatment of subclinical *S. aureus* mastitis. The model may underestimate treatment benefits, because we assumed the costs of penalties for antibiotic residues or high SCC in bulk tank milk and costs of impaired fertility due to mastitis to be zero. If such costs were included in the model, the net profit of treatment vs. no treatment would increase. In addition, we assumed that milk with antibiotic residues is discarded. In the Netherlands, feeding of waste milk is discouraged

to prevent transmission of diseases such as Johne's disease or salmonellosis to calves. In reality, the majority of farmers do not discard milk with antibiotic residues. Waste milk is fed to calves to reduce short-term costs by saving milk replacer, and potential costs of extra disease in the long term are ignored. If we had assumed that discarded milk was fed to calves and that milk replacer and discarded milk have the same value, there would be no net reduced revenue associated with milk withholding. In this situation, the profitability of treatment would increase by €21.25 for 3-d treatment and by €38.96 for 8-d treatment (Table 2). This would make treatment of subclinical *S. aureus* mastitis more attractive economically, at least in the short term.

The partial budget presented in this paper is based on economic and regulatory conditions in the Netherlands, which are very different from those in countries outside the European Union. For example, cost of labor was assumed to be zero in the Dutch model because most farms in the Netherlands are family-owned and operated. Average herd size in the Netherlands is 65 cows. In the northeastern United States, some farms have similar size, ownership, and management. By contrast, large dairy farms in the United States use hired labor for which cost cannot be set at zero. The cost of labor would decrease the benefit of *S. aureus* treatment. In the United States and Canada, a quota system is not in place so that the value of lost milk production is higher than in the Dutch situation. The higher value of lost milk production associated with high SCC in infected animals would increase the calculated prevented losses and hence the benefit of treatment. Feeding waste milk to calves is common practice in the United States and would decrease losses associated with treatment such that the benefit of treatment would increase compared with our estimates. The cost of bacterial culture in New York State is similar to the cost of bacterial culture in the Netherlands, but culture in New York State does not routinely include PS testing. Milk prices differ by country and region and are much higher in the European Union than in some other countries, e.g., Australia and New Zealand. Average feed costs and milk yields differ between countries and breeds. Clearly, the estimates from our model do not apply to all dairy systems. However, our model is an example of a rational approach to decision-making based on scientific knowledge of infection biology and economic considerations. Profit margins in the dairy industry are decreasing. As a result, economic considerations will become increasingly important in decisionmaking. Decision-making based on economic criteria requires a shift in attitude for many producers and veterinarians who have traditionally based decisions on expectations with respect to bacteriological cure or even on emotional considerations. Producers, veterinarians, and herd consultants should shift from the current focus on a high probability of treatment success for individual cows as determined by bacteriological cure to economic consequences of treatment of specific cows on a specific farm.

The current economic model does not cover potential negative side effects of lactational treatment. When use of antibiotics for treatment of subclinical infections is promoted, increased use of antibiotics and an associated risk of increased antimicrobial resistance in *S. aureus* would be anticipated. However, if treatment of subclinical mastitis prevents clinical mastitis and transmission of *S. aureus* to other cows, use of antimicrobials for treatment of subclinical infections may result in a net reduction in antimicrobial usage in the long term. Even when antibiotic usage is not decreased, it is difficult to predict how antimicrobial usage will affect the

prevalence of antimicrobial resistance. Penicillin-based antibiotics have been used widely for decades, and in many studies, a decrease in penicillin resistance among *S. aureus* rather than an increase has been documented in the past decade (Erskine et al., 2002; Sol, 2002; Makovec and Ruegg, 2003). Thus, field data do not justify rejection of antibiotic treatment of subclinical infections based solely on the fear of a potential impact on resistance. The impact of treatment on antimicrobial resistance in *S. aureus* should be monitored, irrespective of the treatment indication (subclinical mastitis, clinical mastitis, dry cow treatment), as has been done and continues to be done in many countries.

CONCLUSIONS

The economic benefit of antibiotic treatment of subclinical mastitis caused by *S. aureus* is dependent on local economic conditions and on host, pathogen, and management factors. On farms where transmission of *S. aureus* is likely, antibiotic treatment of cows with subclinical *S. aureus* mastitis is often profitable, if the right cows are selected for treatment using known risk factors for cure. On farms where contagious transmission is unlikely, extended treatment is nearly always uneconomical, but 3-d antibiotic treatment can be economically profitable, especially for young animals with recent infections with penicillin-sensitive strains of *S. aureus*.

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CHAPTER 4

Efficacy of extended cefquinome treatment of clinical *Staphylococcus aureus* mastitis

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ABSTRACT

Clinical *Staphylococcus aureus* mastitis is difficult to cure. Extended antimicrobial treatment is often advocated as a practical approach to improve cure rates; however, scientific evidence of this hypothesis is lacking. A multi-centered, nonblinded, randomized, positive-controlled clinical trial was conducted in 5 European countries—France, Hungary, Italy, the Netherlands, and the United Kingdom—to study the efficacy of an extended intramammary cefquinome treatment (5 d) compared with a standard intramammary cefquinome treatment (1.5 d) of *Staph. aureus* clinical mastitis. Least squares means estimates of bacteriological cure during lactation were 34% [standard error (SE) = 9.9%] for the standard treatment group and 27% (SE = 8.4%) for the extended treatment group. In the final model, extended therapy was not significantly better. The only factor predicting bacteriological cure was pretreatment cow somatic cell count (SCC). Cows with >250,000 cells/mL in milk before treatment were less likely to cure. Least squares means of clinical cure during lactation was 60% (SE = 19%) for the standard treatment group and 82% (SE = 12%) for the extended treatment group. In the final model, clinical cure after extended treatment was significantly better. Pretreatment cow udder firmness predicted clinical cure. Firm udders were less likely to cure clinically. Irrespective of treatment regimen, new infection rates with pathogens other than *Staph. aureus* were higher (42%) after bacteriological cure than after non-bacteriological cure (22%) and cured cows had a significantly lower SCC. In conclusion, independent of the treatment protocol, cows with an SCC <250,000 cells/mL before treatment showed a higher probability of bacteriological cure. It appears that successful treatment of clinical *Staph. aureus* mastitis with cefquinome is associated with an increased number of new infections with coagulasenegative staphylococci. Extended treatment improved clinical, but not bacteriological, cure rates compared with the standard treatment. These results indicate that extending treatment of clinical *Staph. aureus* mastitis with cefquinome should not be recommended.

Keywords: dairy cow, lactation, mammary gland, antimicrobial

INTRODUCTION

Contagious mastitis has been successfully controlled in recent decades on many farms in many countries due to the large scale adoption of the 5-point plan: optimization of milking and milking machine, blanket dry cow treatment, postmilking teat disinfection, treatment of clinical mastitis, and culling of chronically infected cows (Neave et al., 1969). As a result, the incidence of contagious pathogens, such as *Streptococcus agalactiae* and *Staphylococcus aureus*, has declined, resulting in a dramatic decrease of the average bulk milk SCC (**BMSCC**) in the majority of European countries. Despite this, *Staph. aureus* remains an important mastitis-causing pathogen on many farms, even when BMSCC is low (Barkema et al., 1998; Olde Riekerink et al., 2010).

An important reason the prevalence of *Staph. aureus* remains high in herds is the degree of difficulty to cure existing infections, leading to both repeat clinical episodes and spreading of bacteria to herd mates. Bacteriological cure (**BC**) of *Staph. aureus* mastitis during lactation is generally low, both for subclinical (Sol et al., 1997) and clinical cases (Sol et al., 2000; Bradley and Green, 2009), and is mainly dependent on the duration of infection, the bacterial strain, and the duration of treatment (Barkema et al., 2006). Also, some *Staph. aureus* strains have a stronger clinical manifestation than others (Zadoks et al., 2000; Haveri et al., 2005; Fournier et al., 2008), a lower persistence, and a higher probability of cure (Haveri et al., 2005).

Subclinical *Staph. aureus*-infected cows can be selected for treatment based on their estimated probability of cure. In *Staph. aureus* clinical mastitis (**CM**), however, treatment is necessary to fight the clinical symptoms and to bring the cow back into production as soon as possible. Increased duration of treatment is often used in practice because it is an easy way to possibly improve the probability of cure. Research showing the beneficial effect of extended antimicrobial treatment of subclinical *Staph. aureus* mastitis during lactation are numerous and conclusive (Gillespie et al., 2002; Oliver et al., 2004; Deluyker et al., 2005). However, studies of extended antimicrobial treatment of clinical *Staph. aureus* mastitis are limited and less conclusive, showing a better effect (Jarp et al., 1989), only a numerical difference (Pyorala and Pyorala, 1998), or a significantly higher cure only for β-lactamase negative *Staph. aureus* cases (Sol et al., 2000). Thus, more research on the effects of extended treatment is needed for clinical *Staph. aureus* mastitis.

Cefquinome is a broad-spectrum cephalosporin which is commonly used for the intramammary (**IMM**) treatment of CM throughout Europe, where it has been approved for the treatment of CM caused by the major mastitis-causing pathogens, including *Staph. aureus*. The objective of this study is to compare different aspects of the efficacy of a standard versus an extended duration of IMM cefquinome treatment against clinical *Staph. aureus* mastitis.

MATERIAL AND METHODS

Study Design

This was a multi-centered, nonblinded, randomized, positive controlled clinical trial that was conducted in 5 countries: France, Hungary, Italy, the Netherlands, and the United Kingdom. The protocol was in accordance with the note for Guidance on statistical principles for veterinary clinical trials (EMEA, 2002), the legal requirements in the countries where it was implemented, and the European Guideline for the conduct of efficacy studies for intramammary products for use in cattle (EMEA, 2003).

Lactating cows with clinical *Staph. aureus* mastitis from different herds were randomly assigned to 2 treatment groups, receiving either a standard 1.5-d or an extended 5-d IMM cefquinome treatment. Milk samples were taken around 14 and 21 d after the last treatment to determine the primary efficacy criteria, BC, and clinical cure (**CC**).

Animals and Herds

No specific animal management or housing was required for the selection of herds. Herd-specific data, such as herd size (number of dairy cows), herd management (housing, bedding, feeding), or udder health management (average milking interval, teat disinfecting practices, that is, pre- and postdipping and blanket, selective or no dry cow treatment), were recorded, as well as the 3 most recent BMSCC.

Criteria for Selection of Clinical Mastitis Cases

Lactating cows with CM in a single quarter were included in this study. Clinical mastitis was defined as a quarter with clinical signs (swelling, heat, pain) or any changes in the appearance of milk, with or without associated general clinical signs. For the included cows, historic cow data, such as breed, date of birth, number of lactation, date of last calving, estimated or measured milk yield at time of treatment, cow SCC, and history of previous mastitis cases, were recorded. Excluded were cows with CM occurring less than 30 d from the planned day of drying-off, cows with severe systemic signs needing other systemic treatment, cows with other intercurrent diseases at the time of CM, cows given systemic or IMM anti-inflammatory or antimicrobial treatments within a 30-d period before inclusion, cows with visible teat damage, and cows with daily milk yield less than 5 kg before the onset of clinical signs.

Postadmission Withdrawal

A cow was withdrawn after enrolment if the pretreatment milk sample was contaminated (>2 bacteria species) or if no *Staph. aureus* was isolated. If 2 bacteria were cultured pretreatment, *Staph. aureus* had to be the primary pathogen, having equal or more bacteria than the other pathogen based on semiquantitative analysis (low/moderate/high), to keep the cow in the study.

Also withdrawn were cows experiencing an adverse event, injury, or illness, including mastitis in another quarter or treatment with another antimicrobial or anti-inflammatory. If any other significant deviation from the protocol occurred, the corresponding case was also withdrawn from the study.

Number of Animals

The tested hypothesis was that the extended treatment resulted in a higher cure rate (50%) than the standard treatment (25%). Based on a one-sided, Chisquared test with type I error $\alpha = 0.05$ and type II error $\beta = 0.20$, the estimated sample size is 48 cows per treatment group. Assuming 70% bacteriological positive and noncontaminated milk cultures, a *Staph. aureus* prevalence among these of 10%, and 10% exclusion for adverse events and major deviations, a total of approximately 1,500 cows should be included to reach the target sample size. However, during the study it became clear that the actual prevalence of *Staph. aureus* in noncontaminated bacteriological-positive cultures was much higher than estimated (not 10% but 17.7%); thus, the trial could be stopped before reaching the 1,500 included cows. Finally, 1,217 cows with CM were screened.

Treatment and Randomization

Eligible cows were treated IMM in the infected quarter with 75 mg of cefquinome sulfate (Cobactan LC, MSD Animal Health, Boxmeer, the Netherlands) according to one of the following treatment protocols: extended treatment group = 3 infusions of one tube at 12-h intervals, followed by 3 infusions of one syringe at 24-h intervals (5 d); or standard treatment group = 3 infusions of one tube at 12-h intervals between each infusion (1.5 d). To ensure the homogeneity of treatment groups, cows were allocated randomly to either the extended or the standard treatment group. A randomization list was established in advance for each country using SAS software (version 9.1; SAS Institute Inc., Cary, NC). Each investigator received a set of numbered therapeutic units containing either 6 or 3 tubes. When a new animal was enrolled, the investigator had to give the therapeutic unit with the lowest number as indicated on the randomization list. Any adverse event, whether or not considered treatment related, was recorded and thoroughly reviewed.

Milk Sampling

At inclusion, milk sampling for bacteriology was performed from the affected quarter of all clinical cases. First and second post-treatment quarter milk samples were only taken if *Staph. aureus* was cultured from the sample at inclusion. The first post-treatment milk sample was collected 14 to 17 d after the last IMM infusion, and the second was taken 7 to 10 d later. Therefore, in the extended treatment group, the first post-treatment milk sample was taken between d 18 and 21 and the second post-treatment sampling was taken between d 25 and 31. In the standard treatment group, the 2 post-treatment samplings were between d 15 and 18 and between d 22 and 28, respectively.

Quarter milk sampling was done by the farmer, a technician, or the local investigator from the foremilk before milking, after discarding 3 to 5 streams of milk, according to a strict hygiene protocol. All samples were kept cool (1–8°C) before transport, at ambient temperatures during transport, and kept cool again upon arrival and during analysis in a centralized laboratory (IDEXX, Alfortville, France). In case the shipment was delayed (i.e., sample taken during the weekend), samples were frozen until transport. Two quarter milk samples for SCC were taken, 1 at inclusion and 1 just after the second post-treatment milk sample for bacteriology. At least 2 mL of milk were collected, identified properly, and sent to the laboratory.

Laboratory Procedures

Milk samples were plated with an inoculum size of 0.1 mL (Walker et al., 2010) and bacterial colonies were identified according to National Mastitis Council guidelines (NMC, 1999). Also, semiquantitative analysis (low/moderate/high) of the isolated bacterial species was performed.

A milk sample was considered contaminated if 3 or more bacterial species were isolated. In case of a contaminated sample at inclusion, the animal was withdrawn from the study. If one other bacterial species, besides *Staph. aureus*, was isolated as well, the additional bacterium was identified.

To determine cefquinome resistance in vitro, all the *Staph. aureus* strains isolated from pretreatment milk samples were shipped to MSD Animal Health Reasearch and Development for the determination of their MIC of cefquinome. The MIC were determined using the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) document M31-A3 (CLSI, 2008). Additionally, the production of β-lactamase by the *Staph. aureus* strains was investigated by the nitrocefin disc-based test (Cefinase disc, Becton Dickinson, Franklin Lakes, NJ). All SCC determinations were performed using a DeLaval (Tumba, Sweden) cell counter. If the SCC of a sample could not be determined due to clotting in the milk, a value equal to the upper limit of quantification of the method (4,000 × 1,000 cells/mL) was used for calculations.

Clinical Examination

At inclusion and after the first and second posttreatment milk sampling, a clinical examination was performed by the local investigator. Rectal temperature, general condition, udder swelling (assessed by observation), udder firmness and induration (assessed by palpation), udder pain (assessed by the cow's reaction), and milk quality (assessed by observation) were scored on a clinical examination form ranging from normal (score 0), slightly (score 1), moderately (score 2), and severely affected (score 3). At least 7 d were allowed between the first and second post-treatment clinical examination.

Definitions

Bacteriological cure was defined as the absence of *Staph. aureus* in both post-treatment milk samples. The presence of *Staph. aureus* in one or in both of the posttreatment milk samples was considered a treatment failure. In case 1 or 2 of the post-treatment samples were non-interpretable (>2 pathogens in a sample or noncompliance to the treatment protocol), the case was excluded from BC analysis.

A new infection was defined as the presence, in 1 or 2 of the post-treatment samples, of a pathogen other than *Staph. aureus* and a pathogen other than any pathogen isolated pretreatment. Clinical cure was defined as a cow in normal condition having no clinical sign of mastitis in milk quality or udder aspects (firmness, swelling, pain) at the first post-treatment evaluation. The presence of any clinical sign in any clinical parameter at the first post-treatment sample was considered a clinical treatment failure. Quarter SCC cure was defined as an SCC of ≥200,000 cells/mL of the clinically affected quarter at inclusion that was reduced to <200,000 cells/mL in the second post-treatment sample.

Statistical Analysis

All data were reviewed by the study supervisor to check for clinical consistency. They were entered electronically by an automatic system of data lecture (FORMS 5.2; ReadSoft, Helsingborg, Sweden). All the electronically entered data (100%) used for evaluation of BC were checked against raw data, and 10% of electronically entered data of the CC and SCC were checked against the raw data.

Bacteriological culturing was analyzed using a mixed model logistic regression analysis. For all regression models the linear predictor was given by

Logit (cure) = lactation + DIM + herd size + BMSCC + pretreatment SCC + *Staph. aureus* MIC + *Staph. aureus* CFU + *Staph. aureus* Betalact + treatment + herd (random) + e,

where cure is either CC or BC; lactation is in 3 categories (1, 2, and 3+); DIM is in three 100-d categories; herd size is in categories of 100 animals; BMSCC is the BMSCC before treatment; pretreatment SCC is the cow SCC before treatment; *Staph. aureus* MIC is the MIC value at 0.25, 0.5, 1, or 2 µg/mL; *Staph. aureus* CFU is an indicator for cfu of *Staph. aureus* at the initial sampling; *Staph. aureus* Betalact is an indicator variable indicating the presence or absence of β-lactamase; and treatment indicated extended or standard duration of treatment. All of these factors were included as a fixed effect, and e was a binomial error term. β-Lactamase production of *Staph. aureus* is included in the model because it is a potential confounder due to its association with a lower probability of cure (Osterås et al., 1999; Sol et al., 2000). A random herd effect was included in the model with herd as a unique herd identifier. The CC model also included the results of the clinical investigation at the time of diagnosis. The clinical investigation included udder firmness, swelling, pain, milk characteristics, body temperature, and cow attitude. A backward, stepwise regression analysis was performed. In the final model, all 2-way interactions were evaluated. For all outcome variables, treatment was compared with the control group with significance defined as $P > 0.05$.

RESULTS

Enrolled Cows

The cows in the study were from a diverse population. They were from 5 different European Union countries and originated from 68 different herds with a wide range of geometric BMSCC (237,000–520,000 cells/mL) and clinical *Staph. aureus* incidence (Table 1). A total of 1,217 cows with CM were sampled pretreatment for bacteriological examination. In 646 milk samples other bacteria than *Staph. aureus* were cultured, 286 samples showed no growth, and 70 samples were contaminated, leaving 215 cows with confirmed clinical *Staph. aureus* mastitis. This corresponds to an average prevalence of *Staph. aureus* of 17.7% among CM samples (215/1,217). After inclusion, another 9 cows were withdrawn from the study due to noncompliance to the study protocol. A total of 206 clinical *Staph. aureus* mastitis cases were included, 161 containing *Staph. aureus* only and 45 mixed cultures containing an additional pathogen. Additional pathogens to *Staph. aureus* were *Streptococcus uberis* (16), esculine-positive streptococci (9), *Streptococcus dysgalactiae* (6), CNS (4), Pseudomonas spp. (4), *Escherichia coli* (2), Enterobacteriaceae spp. (2), *Klebsiella pneumonia* (1), and *Arcanobacterium pyogenes* (1). Finally, these 206 included cases, 114 in the extended treatment group and 92 in the standard treatment group, completed the study and were included in the statistical analysis (Table 1).

Homogeneity of Treatment Groups

The data of the 2 treatment groups were compared and tested statistically with a Student's *t*-test or a Kruskal-Wallis one-way ANOVA for homogeneity (Table 2). No significant differences ($P > 0.05$) were found between the age, parity, DIM, milk production, BW, CM history, cow SCC, rectal temperature, or β-lactamase production between the extended and the standard treatment group.

Antimicrobial Susceptibility In Vitro

The MIC of cefquinome for the isolated *Staph. aureus* strains were distributed over a narrow range, from 0.25 to 2 µg/mL, with 98% of the strains (209/213) having an MIC <1 µg/mL. None of the strains was resistant to cefquinome. For most of the countries the MIC50 was 0.5 µg/mL, except Italy, where it was one dilution step higher (1 µg/mL). Despite this slight difference, strains collected in Italy had a good susceptibility against cefquinome, with 96% of the strains (60/64) having an MIC <1 µg/mL.

Table 1. Description of country-specific traits and total traits

	Country[1]					
	FR[1]	**HU**	**IT**	**NL**	**UK**	**Total**
No. of herds	35	8	14	4	7	68
Herd size[2]	45	385	135	67	135	
BMSSC[3] (x 1000 cells/mL)	309	520	313	237	256	
Total of clinical cases (No.)	349	184	433	94	157	1217
S. aureus CM[4] (No.)	53	58	64	28	12	215
S. aureus CM analyzed (No.)	50	58	63	24	11	206
Extended treatment (No.)	30	33	32	14	5	114
Standard treatment (No.)	20	25	31	10	6	92
BC[5] extended, No. (%)	8 (31)	2 (8)	13 (52)	7 (58)	0 (0)	30 (33)
BC standard, No. (%)	2 (11)	4 (18)	13 (61)	7 (62)	0 (0)	25 (33.3)
New infections, No. (%)						47/166 (28)
After BC, No. (%)						23/55 (42)
After non-BC, No. (%)						24/111 (22)
CC[6] extended, No. (%)	20 (67)	7 (24)	31 (100)	14 (100)	3 (75)	75/108 (69.4)
CC standard, No. (%)	9 (45)	4 (18)	29 (96.7)	7 (88)	3 (75)	52/84 (61.9)
QSCC[7] cure extended (%)						27/88 (30.7)
QSCC cure standard (%)						17/72 (23.6)
B-lactamase positive, No. (%)	31 (59)	23 (41)	30 (48)	2 (7)	4 (33)	92 (43)

[1] FR =France, HU = Hungary, IT = Italy, NL= The Netherlands, UK= The United Kingdom.
[2] Median herd size.
[3] Bulk Milk Somatic Cell Count, geometric mean.
[4] Clinical mastitis.
[5] Bacteriological cure.
[6] Clinical cure.
[7] Quarter somatic cell count cure, clinically affected quarters at inclusion that had a QSCC < 200,000 cells/mL after the second post treatment sample.

Table 2. Homogeneity of data between the two treatment groups[1]

Item	**Standard**	**Extended**	**P-value**
Age (years)	5.2 (2.0)	4.9 (2.1)	0.346
Parity	2 (2-4)	2 (2-4)	0.433
Days in milk (d)	149.8 (87.3)	147.2 (93.6)	0.856
Milk production (kg)	26.7 (8.4)	26.4 (8.4)	0.824
Bodyweight (kg)	649.4 (71.0)	646.5 (73.4)	0.784
CM[2] history (No.)	1 (0-2)	1 (0-1)	0.834
Log cow SCC	6.1 (1.4)	6.0 (1.4)	0.648
Rectal temperature (℃)	38.6 (0.4)	38.6 (0.4)	0.957

[1] Most parameters were tested by Student's *t*-test and are shown as the mean and the SD in parentheses. For parity and clinical mastitis history, a Kruskal-Wallis one-way ANOVA was performed to better describe the data, these are shown as medians with their 25% and 75% intervals, respectively, in parentheses.
[2] Clinical mastitis.

Table 3. Final mixed model logistic regression model of bacteriological cure

Effect	Estimate	SE	df	*t*-value	*P*-value	Odds ratio	95% CI
Intercept	2.2196	1.3325	15	1.67	0.12		
Extended treatment							
Yes vs. no	-0.4247	0.4457	108	0.95	0.34	0.66	0.27-1.67
Days in milk[1]							
0-100 days	-0.8261	0.5882	108	-1.40	0.16	0.44	0.14-1.41
101-200 days	-0.7820	0.5761	108	-1.36	0.18	0.46	0.15-1.43
>200 d reference							
Parity[1]							
1	-0.3854	0.6060	108	-0.64	0.53	0.68	0.21-2.26
2	-0.6280	0.5378	108	-1.17	0.25	0.53	0.18-1.56
3+ reference							
Pretreatment							
Log SCC	-0.8657	0.4189	108	-2.07	0.041	0.42	0.18-0.97

[1] Days in milk and parity were kept in the model because they acted as confounders

BC

Twenty-three clinical cases in the extended treatment group and 17 cases in the standard treatment group were excluded from the analysis of BC due to noninterpretable bacteriological results, leaving 91 (114−23) cases in the extended treatment group and 75 (92−17) in the standard treatment group. The overall BC showed no difference between treatment groups: 33.0% (30/91) after extended treatment and 33.3% (25/75), after standard treatment (Table 1). Least squares means estimates for BC of *Staph. aureus* infections during lactation were 34% (SE 9.9%) for the standard treatment group and 27% (SE 8.4%) for the extended treatment group. Logistic regression results of BC are shown in Table 3. In the final model, extended therapy was not significantly different from the control treatment [odds ratio (**OR**) = 0.66, 95% CI (0.27–1.67)]. At the country level, BC also showed no difference per treatment group, except for France, where the cure after extended treatment was significantly better (31%) than after the standard treatment (11%; Table 1). Bacteriological cure at the country level showed a wide variety, with low cure rates in Hungary and the United Kingdom, ranging from 0 to 18%, and high cure rates in Italy and the Netherlands, ranging from 52 to 62%.

Both DIM and parity were nonsignificant risk factors for BC (Table 3). The only significant predictor variable was the pretreatment cow SCC; a higher pretreatment cow SCC indicated a lower probability of cure (OR = 0.42, 95% CI [0.18–0.97]). None of the herd-level variables indicated in the Material and Methods section were statistically significant. Parity was maintained in the final model as it acted as a confounder. Removing lactation number from the model did not affect the significance of the treatment variable. The random effect for herd was estimated at 1.1 (SE 0.78) and was not statistically significant. The overall percentage of β-lactamase-producing *Staph. aureus* strains was 43%, but varied widely between countries, from a noticeably

low percentage of 7% in the Netherlands to 59% in France (Table 1).

Irrespective of treatment protocol, the BC for β-lactamase-positive strains was lower (28.8%) than for β-lactamase-negative strains (36.6%), although the difference was not significant (Chi-squared, $P = 0.29$). Extended treatment did not have an effect compared with standard treatment within β-lactamase-negative (35.8 and 37.5%, respectively) nor within β-lactamasepositive (28.9 and 28.6%, respectively) *Staph. aureus* strains.

CC

Six cases were excluded from the analysis of the CC in the extended treatment group; 4 cases related to mastitis observed in another quarter, 1 case related to missing data, and 1 case that was noncompliant with the treatment protocol. Eight cases were excluded in the analysis of the CC in the standard treatment group; 3 cases related to mastitis observed in another quarter, 1 case related to missing data, and 4 cases were noncompliant with the treatment protocol. One-hundred and eight cases (114–6) were left for analysis in the extended treatment group and 84 (92–8) in the standard treatment group.

The CC in the standard versus the extended treatment groups was 61.9 (52/84) and 69.4% (75/108), respectively (Table 1). Logistic regression results of CC of *Staph. aureus* mastitis during lactation were 60% (SE 19%) for the standard treatment group and 82% (SE 12%) for the extended treatment group. The final model for CC is shown in Table 4. In the final model, CC after extended treatment was significantly better compared with the standard treatment (OR = 3.03, 95% CI [1–9.17]). The only significant predictor variable for CC was the pretreatment cow udder firmness (OR = 0.40, 95% CI [0.20–0.79]). A firm udder at palpation at the time of diagnosis of CM predicted a lower probability of CC. None of the herd-level variables were statistically significant.

Table 4. Final mixed model logistic regression model of clinical cure

Effect	Estimate	SE	df	*t*-value	*P*-value	Odds ratio	95% CI
Intercept	2.9376	1.0879	16	2.70	0.016		
Extended treatment							
Yes vs. no	1.1082	0.5166	14	-2.15	0.050	3.03	1 – 9.17
Parity[1]							
1	-0.4102	0.7043	23	-0.58	0.57	0.66	0.16 – 2.85
2	-1.3802	0.6376	23	-2.16	0.041	0.25	0.07 – 0.94
3+ reference							
Days in milk[1]							
0-100 days	0.000302	0.6385	25	0.00	0.9996	1	0.27 – 3.7
101-200 days	0.5725	0.6829	25	0.84	0.41	1.78	0.43 – 7.24
>200 d reference							
Udder firmness							
Yes vs. no	-0.9256	0.3494	136	-2.65	0.0090	0.40	0.20 – 0.79

[1] Parity and DIM were kept in the model because they acted as confounders

The DIM and parity categories were maintained in the final model because they acted as a confounder. Removing the lactation number from the model did not affect the significance of the treatment variable. The random effect for herd was estimated at 7.1 (SE 4.4) and was borderline statistically significant. Further evaluation of herds indicated that herds in one country (Hungary) had a marked lower CC rate than the other countries (Table 1).

SCC

The overall median SCC in the clinically affected quarters at 21 to 27 d after the last treatment was lower for the 5-d treatment (1,233,000 cells/mL) than for the 1.5-d treatment (2,076,000 cells/mL), but the difference was not significant due to the large variance. The median SCC of the bacteriologically cured quarters (33.5%), for 5-d and 1.5-d treatment was low (112,000 and 130,000 cells/mL, respectively), when compared with nonbacteriologically cured quarters (66.5%; 2,520,000 and 2,954,000 cells/mL, respectively). No difference in quarter SCC was found between treatment groups. The percentage of quarters with an SCC <200,000 cells/mL (quarter SCC cure) after treatment was low: 30.7% after 5-d treatment and 23.6% after 1.5-d treatment (Table 1), which was 3 to 10% lower than BC.

New Infections

The percentage of new infections was comparable and therefore nonsignificant between the treatment groups: 28.6% (26/91) after the extended treatment and 28% (21/75) after the standard treatment (Table 1). Interestingly, irrespective of treatment protocol, the percentage of new infections was much higher for bacteriologically cured quarters (42% [23/55]) than for the noncured quarters (22% [24/111]; Table 1). The majority (around 40%) of the new infections were caused by CNS irrespective of treatment protocol.

DISCUSSION

This study showed that standard IMM treatment of clinical *Staph. aureus* mastitis with cefquinome resulted in a BC rate of 33.3%. This BC rate is comparable to the standard treatment cure rates of clinical *Staph. aureus* mastitis found in the study of Bradley and Green (2009; 31.9%), Pyorala and Pyorala (1998; 29%), Jarp et al. (1989; 38.8%), and Sol et al. (2000; 38%, β-lactam-positive strains) but lower than the 48% (β-lactam-negative strains) cure rates found in the study of Sol et al. (2000) after standard treatment. However, a comparison of cure rates between trials has to be done with care because of different definitions for cure or different conditions in different areas. As in our study, the study of Bradley and Green (2009) was performed in multiple European countries (United Kingdom, Germany, and France), whereas the study of Sol et al. (2000) was performed in a more restricted area (the eastern part of the Netherlands), suggesting that local results cannot always be extrapolated to larger geographic areas. Also, differences were observed in our study in cure rates between countries. The problem

in extrapolating outcomes of *Staph. aureus* treatment studies from one geographical area to another can be caused by many factors, such as differences in *Staph. aureus* strains. This was confirmed by the large differences in the prevalence of β-lactamase-producing *Staph. aureus* strains between countries (Table 1).

Our finding that extended treatment of clinical *Staph. aureus* did not result in an improved overall BC cure compared with standard treatment is in contrast with reports from studies on clinical (Jarp et al., 1989; Pyorala and Pyorala, 1998; Sol et al., 2000) and subclinical *Staph. aureus* mastitis (Gillespie et al., 2002; Oliver et al., 2004; Deluyker et al., 2005) that describe an increased cure after extended treatment. Based on these previous studies, it seems that extended treatment is the best choice when treating *Staph. aureus* mastitis (Roy et al., 2009). Including high BMSCC *Staph. aureus* problem farms, because such farms are more interested to participate in the study, increased the probability of isolating *Staph. aureus.* On this type of farms, infection pressure of *Staph. aureus* is relatively high, possibly resulting in more reinfection of initially cured cows, which, in case the reinfection was with *Staph. aureus*, were recorded as noncured.

We found a much higher new infection rate after BC (42%) when compared with non-BC (22%; Table 1), confirming an increased quarter sensitivity for a new IMI after cure, as previously shown by Zadoks et al. (2001). We hypothesized that, to prevent new infections after cure, extended treatment should be restricted to farms with low infection pressure, likely corresponding to good udder health management, as has been previously suggested in a theoretical model by Barlow et al. (2009). Further research is needed to substantiate this hypothesis in the field. Interestingly, the majority (± 40%) of the new infections after treatment were caused by CNS, which is similar to the findings of Bradley and Green, (2009) and Sampimon et al. (2010), who reported that CNS becomes more important on farms with low BMSCC and a low prevalence of *Staph. aureus*. It seems likely that the absence of *Staph. aureus* or, as in our study, the clearance of *Staph. aureus* by treatment creates a window of opportunity for opportunistic CNS to invade the udder. It has been reported that 30% of the CNS species *Staphylococcus epidermidis* carry the *mecA* gene (Sampimon et al., 2011), much higher than has been reported in *Staph. aureus*. The possible relation between *mecA*-positive CNS and *Staph. aureus* needs to be further clarified.

Additionally, on *Staph. aureus* problem farms where BMSCC is high, *Staph. aureus* infections are not only more prevalent, but also more chronic, resulting in lower cure rates (Barkema et al., 2006). This may explain the low BC rates in Hungary, where the average BMSCC was high (520,000 cells/mL; Table 1). These observations at the bulk tank level are likely to be related to the findings at the individual cow level, where we did find a significantly lower (OR = 0.42, 95% CI [0.18–0.97]) BC in cows with a high pretreatment SCC (≥250,000 cells/mL) compared with cows with a low pretreatment SCC (<250,000 cells/mL) independently of treatment protocol (Table 3). A lack of antimicrobial susceptibility to cefquinome could potentially have contributed to low BC in both treatment groups. Although isolated *Staph. aureus* strains had comparable low MIC levels in all countries, showing good susceptibility in vitro, this does not necessarily correspond to good susceptibility in vivo. Another explanation for the apparent therapy resistance of *Staph. aureus* in vivo is biofilm formation, hindering the antibiotic to reach the site of infection (Melchior

et al., 2006). Also, methicillin-resistant *Staph. aureus* (**MRSA**) caused by the expression of the *mecA* gene is of particular interest due to its susceptibility for β-lactam antibiotics in vitro, but resistance to almost all β-lactam antibiotics in vivo. Cattle are a potential reservoir for MRSA for the human population and, if transferred, can cause infections in humans that are difficult to cure (Holmes and Zadoks, 2011). Reports on MRSA in dairy cattle in different European countries, however, show that the prevalence is relatively low (Hendriksen et al., 2008). A more recent report shows the prevalence of MRSA to be below 2% at the quarter level (Vanderhaeghen et al., 2010). Because we did not test for the *mecA* gene in our *Staph. aureus* isolates, we are not able to determine its prevalence in our study. Potentially, presence of *mecA* resistance could have contributed to a lower bacteriological cure. However, we do not think that *mecA* resistance had a big influence on the comparison in efficacy between treatment groups because of the expected low prevalence of methicillin resistance and the likely equal distribution among treatment groups.

Our study shows an improvement in CC of *Staph. aureus* mastitis after extended treatment compared with the standard treatment. It was surprising to find a difference between CC and BC, as one would expect these parameters to be correlated. We hypothesized that extended treatment reduced the number of *Staph. aureus* cfu to a level at which clinical signs disappeared while leaving enough bacteria for a positive culture. Because we only registered the number of cfu in the milk samples before but not after treatment, this hypothesis could not be evaluated. Another explanation for the discrepancy between CC and BC is reinfection with *Staph. aureus*. Clinical cure was measured 14 d posttreatment, whereas BC was measured both on d 14 and 21 post-treatment. Equal BC and CC at d 14 posttreatment may, after subsequent reinfection with *Staph. aureus* in the 7 consecutive days, result in a relatively lower BC than CC. Of the clinical parameters measured at inclusion, only udder firmness remained in the final model, indicating that a firm udder at palpation at the time of diagnosis of CM predicted a lower probability of CC. The practical implication of this finding for treatment decisions of CM is not clear, although it could be helpful in predicting the resolution of clinical signs after treatment.

Extending exposure of bacteria to antimicrobials potentially increases the selection of resistant bacteria. Because extended treatment only showed an effect on CC, which is based on subjective judgment and not on BC, extending treatment of *Staph. aureus* CM cannot be justified. To minimize bias, this positively controlled field trial should have been double-blinded. This was not possible because the treatment groups had a different number of tubes, which could not be hidden from the investigators and the farmers. As laboratory personnel were unaware of treatment group allocations, the absence of a double-blind design is unlikely to have seriously affected the outcome of the BC. For the CC, however, this was different because the same investigator, who randomly selected the treatment protocol and applied the first tube, could potentially have remembered the treatment protocol while scoring the CC 2 and 3 wk later. Although we cannot exclude bias of the investigators judgment for CC, we assumed that in the majority of cases they did not remember the treatment protocol while judging CC later on.

CONCLUSIONS

In conclusion, independent of the treatment protocol, cows with SCC <250,000 cells/mL before treatment showed a higher probability of bacteriological cure. It appears that successful treatment of clinical *Staph. aureus* mastitis is associated with an increased number of new infections with CNS. Extended treatment of clinical *Staph. aureus* with cefquinome resulted in a higher CC but not BC than did standard treatment. These results indicate that extending treatment of clinical *Staph. aureus* mastitis should not be recommended.

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CHAPTER 5

Effect of extended cefquinome treatment on clinical persistence or recurrence of environmental clinical mastitis

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ABSTRACT

The effectiveness of antibiotic treatment of clinical mastitis (CM) is classically evaluated using bacteriological cure, which provides a concise and objective way of assessing efficacy but does not reflect the situation in the field where persistence or recurrence of clinical signs lead to perceived treatment failure. If clinical signs persist or recur, intramammary (IMM) treatment is often extended or supplemented with parenteral therapy in the expectation of a more efficient elimination of clinical signs or a lower probability of recurrence. The objective of this study was to evaluate the efficacy against clinical persistence or recurrence of three cefquinome treatment regimes, standard 1.5-day intramammary (SIMM), 5-day extended intramammary (EIMM) and combination of EIMM plus 5-day extended parenteral (ECOMBO) treatment.

The study was conducted on three dairy farms with a high recurrence rate of environmental mastitis. Efficacy was evaluated using a multi-level model at the quarter and at the cow level, based on the persistence or recurrence of clinical signs at any time during a 105-day period following the end of the initial treatment, independent of pathogen.

The most prevalent pathogens were *E. coli* (16.9%) and *S. uberis* (11.97%). EIMM and ECOMBO significantly decreased the persistence or recurrence of CM by 8% and 6% at the quarter level and by 9% and 8% at the cow level, respectively. ECOMBO may not reduce the persistence or recurrence of CM beyond EIMM. Whilst extended treatment regimens offered an improved outcome in this study, the producer and practitioner need to carefully consider such regimens from the perspective of prudent antibiotic use.

Keywords: Bovine, Lactation, Mammary gland, Extended treatment, Antibiotic

INTRODUCTION

Environmental pathogens, particularly *Streptococcus uberis* and *Escherichia coli*, can be a cause of persistent intramammary infection (Van Eenennaam et al., 1995; Döpfer et al., 1999; Bradley and Green, 2001). On some farms, with a low bulk milk somatic cell count (BMSCC) and high incidence of clinical mastitis (CM), a significant proportion of CM may occur in a limited number of animals as a result of a high level of recurrence (Houben et al., 1993; Lam et al., 1996; Zadoks et al., 2001). Recurrent CM cases have been described as being as severe as index cases, with comparable impact on milk yield and probability of death (Bar et al., 2007). Moreover, cows with recurrent CM are at a higher risk for culling (Bar et al., 2008).

Recurrent CM is usually defined by initial disappearance and subsequent re-occurrence of clinical signs after a preset number of days. Using this definition, recurrent CM can be due to a recrudescence of a persistent IMM infection due to failure to cure (Pinzón-Sánchez and Ruegg, 2011), or as a result of re-infection of the quarter after successful cure. However, differentiating between persistence of infection and re-infection is not possible in the field. Generally, in practice, the disappearance of clinical signs is considered as a cure, whereas persistence or recurrence of clinical signs is considered as a treatment failure. This treatment failure is what is evaluated in this study.

One of the consequences of successful elimination of the causative bacteria is a shortened timeframe during which infection can spread to other cows in the herd via the milking machine, the milker or the environment. Potentially, improving bacteriological cure rates decreases the infection pressure on healthy cows and so prevents new CM cases. At the same time bacteriological cure also prevents the recrudescence of persistent infections (Van Eenennaam et al., 1995). These indirect effects of cure may play a role in decreasing the overall incidence of CM.

A number of approaches to improve CM bacteriological cure have been evaluated, such as extending treatment duration (Sol et al., 2000; Oliver et al., 2004; Milne et al., 2005) and additional parenteral therapy (Shpigel et al., 1997; Erskine et al., 2002; Wenz et al., 2005). However, such studies have not evaluated the longterm outcome of treatment, nor do they necessarily accurately reflect the field situation where CM treatment outcomes are assessed by the elimination of clinical signs, such as abnormal milk, swelling or redness of the udder. In the field, if clinical signs persist or recur, IMM treatment is often extended or reinstated with parenteral treatment in the expectation of a more effective elimination of clinical signs, leading to the use of additional antibiotic on farm. However, there are few reports on the effects of extended treatment, with or without parenteral treatment, on CM persistence or recurrence.

Clinical mastitis can be treated with different types of antibiotics. Cefquinome is a broad-spectrum β-lactam antibiotic for the treatment of CM, via the IMM and parenteral routes and is licensed as a combination therapy for *E. coli* mastitis in the UK. Concurrent use of IMM and parenteral cefquinome in CM has been evaluated (Shpigel et al., 1997; Ehinger et al., 2006). In herds in which environmental mastitis predominates, the aetiology is necessarily diverse thereby demanding a broad-spectrum antibiotic for first treatment of CM in the absence of previous identification of the causative pathogen.

The aim of this study was to evaluate the effect of different cefquinome treatment regimes in a field based context on the likelihood of clinical persistence or recurrence of CM in dairy herds with high recurrence rates of environmental mastitis.

MATERIALS AND METHODS

Farms

Three commercial dairy farms in Somerset, UK, were selected on the basis of access to electronic records, a history of a high rate of recurrence of CM and a predominance of environmental mastitis (Table 1). CM cases were sampled from August 2009 until November 2010. Monthly milk production, individual cow somatic cell counts (SCC) and all CM cases had been recorded for at least 12 months prior to the start of the study. Milking protocols were comparable between farms, post milking teat disinfection, pre-dipping or pre-wiping and inspection for CM was practiced on all farms in all cows throughout lactation. Milking procedures and equipment did not change during the study period. All three farms used blanket antibiotic dry cow treatment.

Table 1. The characteristics of the three herds involved in the study.

Farm ID	C	H	S
Number of dairy cows	560	239	308
BMSCC (x 1,000 cells/ml)[a]	248	201	158
ICRM[b]	85	116	76
Predominant Housing	Cubicles	Cubicles/pasture	Cubicles
Predominant Breed	HF	HF	HF
Approx 305 Day Yield (L)[c]	9,159	9,003	11,309
Milking Frequency/day	2X	2X	3X

[a] BMSCC= Bulk Milk Somatic Cell Count, 12 months rolling mean,
[b] IRCM= Incidence Rate of Clinical Mastitis (number of quarter cases per 100 cows per year),
[c] HF= Holstein Fresian

Animals

Lactating Holstein Friesian dairy cows with CM in one or more quarters were enrolled. Animal parity, yield, historic SCC, CM history, treatment history and relevant clinical data were recorded contemporaneously onto data capture forms or retrieved from on-farm software.

Inclusion and exclusion criteria

Cows were eligible for the study if they were in good general health and had four functional quarters free from clinically significant udder, teat and teat orifice lesions. Cows were followed for 105 days after treatment and when cows were dried off or removed from the herd earlier, right

censoring was used. Data from animals that were dried off or removed from the herd due to death or culling were analyzed until the day of dry off or removal.

Treatment allocation

Cows were randomly allocated to a treatment group, by the herdspersons based on line numbers. Line numbers were allocated randomly on farm at the moment animals joined the herd. Cows that developed CM were sampled aseptically before treatment, according to their pre-assigned treatment group. When clinical signs did not resolve ('treatment failure') during the 105 day period after the last treatment of an animal's first enrolled clinical case, or if clinical signs disappeared and recurred at any time point during that period, the cows were treated again with the same treatment regime on all subsequent occasions.

Treatment

All treatments were administered by farm personnel and three different regimes were evaluated: (1) 1.5-day IMM treatment with cefquinome 75 mg (Cobactan LC, MSD Animal Health), twice on the first day, at two consecutive milkings and once, at the morning milking on the following day (SIMM); (2) 5-day IMM treatment with cefquinome 75 mg, six times, twice on the first day, at two consecutive milkings, four times once a day, at the morning milking (EIMM); (3) 5-day combination treatment with cefquinome 75 mg IMM, six times, twice on the first day at two consecutive milkings and once, at the morning milking on the following 4 days, plus cefquinome sulphate suspension (1 mg/kg, Cobactan 2.5%, MSD Animal Health) by intramuscular injection five times at 24-h intervals (ECOMBO).

Post admission withdrawal

Animals were withdrawn post admission due to missing data, injury or disability or abnormalities, or concomitant disease or disease other than CM requiring antibiotic or anti-inflammatory treatment.

Detection of CM, persistence of clinical signs and milk sampling

CM was defined as a quarter with any visible change of milk aspect and was identified by farm personnel, who had been trained and assessed in the detection, classification and sampling of CM. Individual cases were assessed for persistence or recurrence of clinical signs at every milking (twice daily on two units and three times daily on one unit). The severity of CM was classified using a three-grade scale: Grade 1, mild (only clots in the milk); Grade 2, moderate (milk aspect changes in colour and/or consistency and/or presence of clots, heat, pain and/or swelling of the udder); and Grade 3, severe (milk aspect changes in colour and/or consistency and/or presence of clots, fever, depression, anorexia, very swollen udder). Any concurrent treatments were also recorded. Prior to treatment farm personnel collected milk samples from affected quarters. Milk samples were frozen (-20°C) and collected for submission to the laboratory on a weekly basis.

Laboratory methods

Microbiological investigation and SCC were carried out using the standard milk sample examination techniques, according to the standard recommended by the International Dairy Federation (Bulletin 132, 1981), International Standard 13366-1:1997 (E) and 13366-2:1997 (G). More specifically, three plates were used and 10 lL of secretion were inoculated onto sheep blood agar and Edward's agar, and 100 lL of secretion were inoculated onto MacConkey agar to enhance the detection of Enterobacteriaceae before incubation at 37 °C. All plates were read at 24, 48, and 72 h. Organisms were identified and quantified using standard laboratory techniques (NMC, 1999; Quinn et al., 1994). E. coli was identified by colony morphology, oxidase, and indole tests; other Enterobacteriaceae were identified using a microtube identification system (RapiD 20 E).

Efficacy of treatment

Treatment was considered effective if clinical signs had resolved after the last treatment and did not recur in the 105 day period after treatment, independent of the bacteria involved. To allow assessment of the potential benefits of systemic treatment on concurrently infected (but not clinically affected) quarters, efficacy was assessed at the quarter and cow level. At the quarter level, lack of efficacy was based on clinical persistence or clinical recurrence of CM in the same quarter. At the cow level, lack of efficacy was based on clinical persistence or clinical recurrence of CM in the same cow, irrespective of the quarter involved.

Data handling and statistical analysis

In this randomized, positive controlled, unmasked, three treatment group study, the null hypothesis was that there was no difference in time to clinical recurrence of CM between groups. This hypothesis was analyzed in a multi-level model. The clinically affected quarter was the experimental unit, with the subsequent analysis taking into account the effect of clustering of cases within quarters, and quarters within cows. Inevitably in studies such as this some cows were allocated to treatment group incorrectly. In order to ensure compliance in a large field based study such as this, farmers were allowed some discretion in individual cow treatment allocation. Analysis explored the impact of deviations from the predefined treatment protocols. Cow and farm data were transferred to a database (Microsoft Access 2003) and all fields were checked for unusual or impossible entries. Data fields were coded as categorical or continuous as appropriate and data transformations carried out for continuous data to normalize distribution, when necessary. The outcome variable of interest was the persistence or recurrence of clinical signs of mastitis after the end of treatment. Initial analysis consisted of descriptive statistics and graphical assessment. Conventional Kaplan–Meier survival curves were constructed to provide a visual display of clinical persistence or recurrence ('treatment failure') of CM. To construct this curve, the 105 day post treatment study period was divided into 7-day blocks. Each case in each block was coded as recurrent or persistent (CM = 1) or not recurrent or persistent (CM = 0) at

the quarter level. Cows were censored at the end of the 105-day follow-up period, at the end of lactation, or after death or culling. Discrete time survival models with random effects were specified so that correlations within the data (cases within quarters and quarters within cows) were accounted for as appropriate in a (frailty) model. The model took the form;

CM_{tijk} ~ Bernoulli probability (mean = μ_{tijk})

$$\text{Logit } (\mu_{tijk}) = \alpha + \log t_{tijk} + \log t_{tijk}^2 + \log t_{tijk}^3 + \log t_{tijk}^4 + \beta_1 X_{ijk} + \beta_2 X_{jk} + \beta_3 X_k + u_{jk} + v_k$$

where t is the week of lactation after previous CM, i, j and k denote the i^{th} CM case in the j^{th} quarter of the k^{th} cow, π_{ijk} the fitted probability of clinical persistence or recurrence of CM after treatment for case i in quarter j of cow k, α the regression intercept, X_{ijk} the vector of covariates at case level, β_1 the coefficients for covariates X_{ik}, X_{jk} the vector of quarter level covariates, β_2 the coefficients for covariates X_{jk}, X_k the vector of cow level covariates, β_3 the coefficients for covariates X_k, u_j the random effect to reflect residual variation between quarters and v_k the random effect to reflect residual variation between cows (both random effects assumed to be normally distributed with mean = 0 and variances Ω_u and Ω_v respectively).

The distributions of covariates were assessed and transformations or re-categorization carried out as deemed appropriate on biological grounds. Model building was carried out using MLwiN with penalized quasi-likelihood for parameter estimation (Rasbash et al., 2010). To avoid the potential biased estimates that can arise from quasi-likelihood methods (Browne and Draper, 2006) final models were selected using Markov chain Monte Carlo (MCMC) for parameter estimation in WinBUGS (Spiegelhalter et al., 2004) using methods described in detail previously (Green et al., 2004). Covariates remained in the model when the 95% credibility intervals for the odds ratios (OR) did not include 1.00. Biologically plausible interactions between significant covariates were tested and included when the 95% credibility intervals for the OR of the interaction term did not include 1.00.

Predictions of the survival time to clinical persistence or recurrence of CM after treatment were made using posterior predictive assessments (Gelman et al., 1996; Green et al., 2007). This incorporates the full model posterior predictive distribution, and was used to evaluate model fit and to illustrate the predicted impact of treatment on time to recurrence of CM. The effects of additional treatments were evaluated statistically by including terms for the extra treatments in multivariate models.

RESULTS

CM occurred in 1008 cases on three study farms, of which 994, mainly mild to moderate cases, were enrolled (Table 2). Fourteen cases (1008-994) were excluded due to missing data. Ninety-three cows were incorrectly allocated to treatment group, 124 received a NSAID concurrently and 106 received additional systemic antibiotics.

Table 2. Severity of clinical mastitis, indicated in numbers of cases and percentages in parenthesis.

Clinical signs	SIMM[d]	EIMM[e]	ECOMBO[f]	Total
Grade 1[a]	175 (57)	192 (60)	222 (60)	589 (59)
Grade 2[b]	109 (36)	100 (31)	125 (34)	334 (34)
Grade 3[c]	21 (7)	26 (8)	24 (6)	71 (7)
Total	305 (100)	318 (100)	371 (100)	994 (100)

[a] Grade 1, mild (only clots in the milk)
[b] Grade 2, moderate (milk aspect changes in colour and/or consistency and/or presence of clots, heat, pain and/or swelling of the udder)
[c] Grade 3, severe (milk aspect changes in color and/or consistency and/or presence of clots, fever, depression, anorexia, very swollen udder)
[d] SIMM, intramammary cefquinome treatment for 1.5 days
[e] EIMM, extended intramammary cefquinome treatment for 5 days
[f] ECOMBO, extended combined intramammary and parenteral cefquinome treatment for 5 days.

Table 3. Aetiology of clinical mastitis cases per treatment group

	Treatment				
Diagnosis (n)	SIMM[a]	EIMM[b]	ECOMBO[c]	Total	Total (%)
E. coli	54	55	59	168	16.90
S. uberis	31	41	47	119	11.97
S. dysgalactiae	14	13	22	49	4.93
S. aureus	11	17	14	42	4.23
Bacillus spp	9	14	14	37	3.72
Yeast spp	14	13	7	34	3.42
Enterococcus spp	10	8	6	24	2.41
Klebsiella spp	6	8	3	17	1.71
Prototheca spp	11	3	1	15	1.51
A. pyogenes	2	1	10	13	1.31
Enterobacter spp	4	2	3	9	0.91
Aerococcus	3	1	4	8	0.80
Pseudomonas spp	2	3	3	8	0.80
Other Major pathogen	8	12	8	28	2.81
Any Enterobacterial involvement	76	83	77	236	23.74
Mixed aetiology (major pathogens)	16	22	30	68	6.84
Corynebacterium spp	17	19	30	66	6.64
Coagulase –ve Staph	12	11	30	53	5.33
Mixed aetiology (minor pathogens)	9	11	13	33	3.32
Contaminated	11	11	8	30	3.02
No growth	61	53	59	173	17.40
Grand Total	305	318	371	994	100.00

[a] SIMM is intramammary cefquinome treatment for 1.5 days,
[b] EIMM is extended intramammary cefquinome treatment for 5 days,
[c] ECOMBO is extended combined intramammary and parenteral cefquinome treatment for 5 days.

These data were included in the statistical analysis and included as covariates in the initial analysis. There was a large variety of pathogens obtained from the samples and they are listed in Table 3. The most frequently isolated pathogens were *E. coli* (16.9%) and *Strep. uberis* (12%). These pathogens can be associated with typical environmental CM etiology which was seen in both first and recurrent cases. Milk production, parity, underlying mastitis pathogens, CM history and treatment history did not differ significantly between groups.

Quarter level

Clinical persistence or recurrence at the quarter level is shown in Table 4. EIMM and ECOMBO treatment reduced the clinical persistence or recurrence of CM by 8% and 6%, respectively (EIMM, OR= 0.38, 95% CI [0.12-0.50] and ECOMBO, OR=0.26, 95% CI [0.19-0.72]). ECOMBO did not further decrease clinical persistence or recurrence when compared to EIMM alone.

Table 4. Numbers and percentages (between brackets) of persistence or recurrence of clinical mastitis at the quarter level within 105 days after the end of treatment of the initial clinical mastitis, irrespective of the isolated bacterial species.

	Treatment			
Recurrence	**SIMM[a]**	**EIMM[b]**	**ECOMBO[c]**	**Total**
No (%)	158 (52)	192 (60)	216 (58)	566 (57)
Yes (%)	147 (48)	126 (40)*	155 (42)*	428 (43)
Total	305	318	371	994

[a] SIMM is intramammary cefquinome treatment for 1.5 days,
[b] EIMM is extended intramammary cefquinome treatment for 5 days,
[c] ECOMBO is extended combined intramammary and parenteral cefquinome treatment for 5 days.
* The odds ratio (OR) of recurrence after EIMM and after ECOMBO treatment were statistically significantly different from the SIMM treatment group (EIMM, OR = 0.38, 95% CI [0.12-0.50] and ECOMBO, OR=0.26, 95% CI [0.19-0.72].

The time to quarter level clinical persistence or recurrence for the three treatment groups is illustrated in Fig. 1. A posterior prediction of treatment effects is shown in Fig. 2, which is comparable to the survival curve in Fig. 1, demonstrating good model fit, illustrates the predicted outcome for the three treatment groups based on the final model. Other significant covariates for quarter level clinical persistence or recurrence were farm, quarter location, parity and NSAID treatment. Infection severity was not a risk for persistence or recurrence (Table 6).

Table 6. Final model outcome of the multivariate analysis of probability of clinical persistence or recurrence at the quarter level

Model term	OR	95% CI	
Intercept = -3.2			
Ref = SIMM[a]			
EIMM[b]	0.26	0.12	0.50
ECOMBO[c]	0.38	0.19	0.72
Ref = farm C[d]			
Farm H	2.58	1.35	5.32
Farm S	0.79	0.36	1.73
Ref = quarter LF[e]			
Quarter LH	0.66	0.35	1.24
Quarter RF	0.49	0.25	0.93
Quarter RH	1.08	0.56	2.09
Ref = Parity 1			
Parity 2	1.31	0.52	3.27
Parity 3	3.23	1.25	8.25
Parity 4	4.74	1.73	13.17
Parity 5+	3.29	1.32	8.26
Ref = Grade 1[f]			
Grade 2	1.04	0.63	1.73
Grade 3	1.62	0.51	5.23
Ref = Yes NSAID[g]			
No NSAID	0.30	0.11	0.74

Ref: reference parameter

[a] SIMM is intramammary cefquinome treatment for 1.5 days,

[b] EIMM is extended intramammary cefquinome treatment for 5 days,

[c] ECOMBO is extended combined intramammary and parenteral cefquinome treatment for 5 days.

[d] For farm characteristics, see Table 1.

[e] LF= left front, RF=right front, LH=left hind, RH=right hind.

[f] Grade 1, Mild; Only clots in the milk, Grade 2, Moderate; Heat, pain and/or swelling of the udder, Grade 3, Severe; Fever, depression, anorexia, very swollen udder.

[g] Yes NSAID = Additional Non Steroid Anti Inflammatory Drug injection.

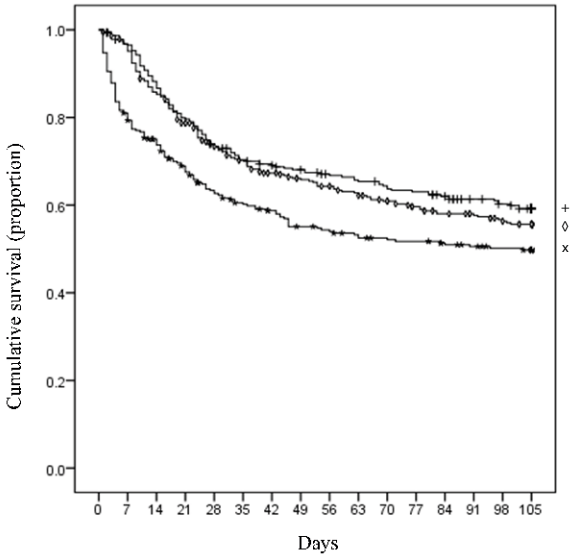

Figure 1. Kaplan Meier survival curve, illustrating cumulative survival (=no persistence or recurrence) at the quarter level, during 105 days after initial treatment for the 3 different treatment groups:

- x line is the short intramammary cefquinome treatment (SIMM) for 1.5 days,
- \+ line is the extended intramammary (EIMM) cefquinome treatment for 5 days,
- ◊ line is the extended combined intramammary and parenteral cefquinome treatment (ECOMBO) for 5 days.

Both EIMM and ECOMBO treatment reduced persistence or recurrence of clinical mastitis significantly compared to the SIMM (EIMM, OR = 0.38, 95% CI [0.12-0.50] and ECOMBO, OR=0.26, 95% CI [0.19-0.72].

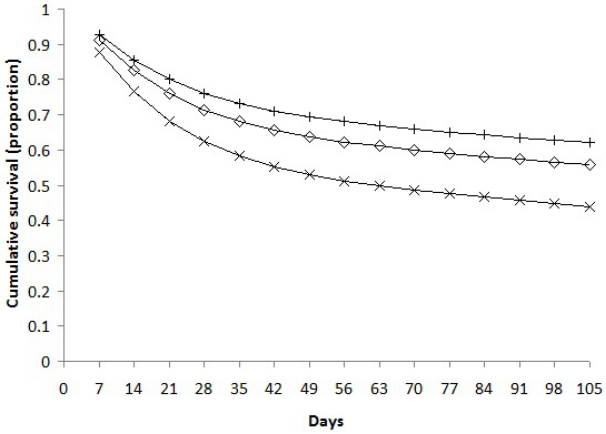

Figure 2. The Bayesian prediction of survival (=no persistence or recurrence) of clinical mastitis after initial treatment from the multilevel model. The prediction is made for every quarter assuming it could receive each treatment:

- x short intramammary cefquinome treatment (SIMM) for 1.5 days,
- \+ extended intramammary (EIMM) cefquinome treatment for 5 days,
- ◊ extended combined intramammary and parenteral cefquinome treatment (ECOMBO) for 5 days.

Cow level

Cow level clinical persistence or recurrence rates are presented in Table 5. The reduction in CM persistence or recurrence was 9% for EIMM and 8% after ECOMBO (EIMM, OR= 0.55, 95% CI [0.38-0.77] and ECOMBO, OR=0.66, 95% CI [0.47-0.93]).

The final multivariate model showed that, apart from treatment regime, the significant covariates for clinical persistence or recurrence of CM at the cow level were farm, quarter location, parity, and *Strep. uberis* infection compared to *E. coli* infection (Table 7).

Table 5. Numbers and percentages (between brackets) of persistence or recurrence of clinical mastitis at the cow level within 105 days after the end of treatment of the initial clinical mastitis, irrespective of the isolated bacterial species

	Treatment			
Recurrence	**SIMM[a]**	**EIMM[b]**	**ECOMBO[c]**	**Total**
No (%)	111 (36)	144 (45)	162 (44)	417 (42)
Yes (%)	194 (64)	174 (55)*	209 (56)*	577 (58)
Total	305	318	371	994

[a] SIMM is intramammary cefquinome treatment for 1.5 days,
[b] EIMM is extended intramammary cefquinome treatment for 5 days,
[c] ECOMBO is extended combined intramammary and parenteral cefquinome treatment for 5 days.
* The Odds Ratio (OR) of recurrence of EIMM and ECOMBO treatment were statistically significantly different from the SIMM treatment group (EIMM, OR= 0.55, 95% CI [0.38-0.77] and ECOMBO, OR=0.66, 95% CI [0.47-0.93].

Table 7. Final outcome of the multivariate model of the probability of clinical persistence or recurrence at the cow level

Model term	**OR**	**95% CI**	
Intercept = -4.0			
Ref = SIMM[a]			
EIMM[b]	0.55	0.38	0.77
ECOMBO[c]	0.66	0.47	0.93
Ref = farm C[d]			
Farm H	1.74	1.22	2.48
Farm S	0.90	0.59	1.34
Ref = Parity 1			
Parity 2	1.80	1.10	3.03
Parity 3	3.10	1.85	5.55
Parity 4	3.31	1.93	6.10
Parity 5+	3.32	2.04	5.71
Ref = *E. coli*[e]			
S. uberis	1.96	1.21	3.20

Ref: reference parameter
[a] SIMM is intramammary cefquinome treatment for 1.5 days,
[b] EIMM is extended intramammary cefquinome treatment for 5 days,
[c] ECOMBO is extended combined intramammary and parenteral cefquinome treatment for 5 days.
[d] For farm characteristics, see Table 1.
[e] Recurrence of clinical mastitis causing pathogen. Recurrence of other bacteria was not significantly different from the reference pathogen, *E. coli*.

DISCUSSION

Bacteriological cure is classically used to assess mastitis treatment efficacy because it is a concise and objective parameter. On farm, treatment efficacy is evaluated based on resolution of clinical signs and lack of recurrence. Persistence or recurrence of clinical signs often results in extended intramammary treatment or additional parenteral treatment, expecting a more efficient elimination of clinical signs and/or a lower probability of recurrence of clinical signs.

In this study, EIMM and ECOMBO were associated with a significant decrease in the probability of persistent or recurrent CM, both at the quarter level and at the cow level when compared to SIMM, in line with other recent findings (Pinzón-Sánchez et al., 2011). This suggests that extended and more 'aggressive' treatment regimens can be beneficial for individual cows when clinical persistence or recurrence rates are high. A reduction in persistent or recurrent CM can be due simply to higher bacteriological cure after extended treatment (Sol et al., 2000, Oliver et al., 2004, Pinzón-Sánchez et al. 2011), though it could also be as a result of a decrease in the risk of re-infection with another pathogen.

Our study differs from earlier studies in that *E. coli* was the most frequently isolated pathogen (Table 3). Döpfer et al., (1999) and Bradley and Green, (2001) used molecular methods to demonstrate that clinical *E. coli* mastitis can recur, and that recurrent *E. coli* strains may be cow adapted. We found that *Strep. uberis* CM was nearly twice as likely to clinically persist or recur as *E. coli* CM. We also found a numerical reduction in clinical persistence or recurrence of *E. coli* CM after EIMM compared to SIMM (data not shown), although the difference was not statistically significant. This is in contrast to studies that show that antibiotic treatment of *E. coli* CM should be avoided because it is not effective (Pyörälä et al., 1998) or not expected to be effective in recurrent cases (Schukken et al., 2004).

An often overlooked, indirect effect of increasing bacteriological cure is a lower infection pressure, simply because cured quarters are no longer able to spread infection to other quarters or other cows (Swinkels et al., 2005a, 2005b; Barlow et al., 2009). This means extended treatment may not only result in a higher bacteriological cure but may also have an indirect effect in a herd, such as an overall lower re-infection rate and thus, less persistence or recurrence of CM. Because treatment strategies were compared within herds, the 'infection pressure' for each treatment group was the same and could not have influenced differences between treatment groups.

Clinical persistence or recurrence of CM was higher at the level of the cow (58%) than the quarter (43%). This was expected, because at the cow level, CM can occur in any of the four quarters. The difference between clinical persistence or recurrence at the cow and quarter level was not large and indicates that the probability of CM recurrence in the other 3 quarters was relatively low and shows persistence or recurrence mainly occurred in the originally affected quarter. This can be either caused by the fact that chronically infected quarters may 'flare-up' after treatment (Houben et al., 1993, Lam et al., 1996, Zadoks et al., 2001) and/or that previously infected quarters are more susceptible to new infection (Zadoks et al., 2001).

Our model was not built to compare ECOMBO and EIMM treatment directly as both were compared to SIMM. However, we believe ECOMBO treatment was not likely to have reduced persistence or recurrence of CM, at the quarter or cow level, beyond EIMM treatment. In contrast, the probability of clinical persistence or recurrence at the cow level was numerically higher in the ECOMBO group than in the EIMM group (Figure 1). However, it is possible that additional parenteral treatment in the ECOMBO group contributed to removal of subclinical infections (with minor pathogens) in the same cow (Sérieys et al., 2005), making those quarters more susceptible to new infections, thereby increasing the likelihood of subsequent CM and recurrence at the cow level. This is in line with the findings of Wenz et al. (2005), who concluded that parenteral treatment with a cephalosporin in addition to standard IMM cephalosporin treatment had no effect on recurrence of mild *E. coli* CM. Our results suggest that ECOMBO treatment with cefquinome may have no added value over EIMM treatment on farms with a high rate of recurrence where environmental pathogens predominate.

For quarters in cows with an additional NSAID treatment, clinical persistence or recurrence was significantly higher (Table 6) compared to quarters in cows where NSAID treatment was not used. This is unexpected, because clinical symptoms are assumed to resolve more quickly after NSAID treatment. It may be that farm personnel used additional NSAID treatment in cows which they suspected clinical symptoms to resolve more slowly or which they perceived to be more sensitive to mastitis and thus more likely to recur.

Prudent antibiotic use is a pre-requisite in modern agriculture and demands evidence-based justification for extended treatment. Exposure of bacteria to antibiotics increases the risk of selection for antibiotic resistance. EIMM treatment led to increased antibiotic use (from three to six tubes per case) and an increase in the duration of exposure to antimicrobials, which was not compensated by the 8-9% decrease in antibiotics used for recurrent cases. Thus, EIMM treatment led to an overall increase in antibiotic exposure, albeit that that exposure was largely confined to the mammary microbiome. The ECOMBO approach clearly increased antibiotic exposure compared to EIMM, as well as resulting in exposure of the gut flora to antimicrobial activity, and did not seem to lower persistence or recurrence of CM beyond EIMM. This study challenges the perception that additional parenteral treatment will improve the outcome of all CM and re-enforces the need for such approaches to only be used for known pathogens. Whilst research suggests the use of parenteral antibiotics in severe mastitis cases may be helpful (Wenz et al., 2001, Erskine et al., 2002), further research is needed to better define the need and/or criteria for the use of systemic antibiotics in the treatment of mild and moderate cases of CM.

In conclusion, both EIMM and ECOMBO cefquinome treatment significantly reduced the persistence or recurrence of CM on farms with a high incidence of mild and moderate environmental mastitis. Because additional extended parenteral treatment beyond EIMM may not reduce clinical persistence or recurrence, the producer and practitioner need to carefully consider such regimes from the perspective of prudent antibiotic use.

Conflict of interest statement

None of the authors has any financial or personal relationship that could inappropriately influence or bias the content of the paper.

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CHAPTER 6

Efficacy of standard versus extended intramammary cefquinome treatment of clinical mastitis in cows with persistent high somatic cell counts

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ABSTRACT

Extended duration of clinical mastitis (CM) treatment has been advocated, although results showing its higher efficacy compared to standard treatment are difficult to compare and seem conflicting. In a non-blinded, positively controlled clinical trial with systematic allocation, the efficacy of a standard, 1.5 day cefquinome treatment (ST), and an extended, 5-day intramammary cefquinome treatment (ET) were evaluated.

The latter is frequently performed in cows with persistent high SCC, expecting a better cure. Therefore, cows with a CM immediately preceded by at least two consecutive monthly elevated SCC > 200,000 cells/ml, were studied. The primary efficacy criteria were bacteriological cure (BC) and clinical cure (CC), while SCC cure was considered a secondary criterion of cure. Least square means of overall BC were not different after ET (79%, n=206) compared to ST (72%, n=203). Extended treatment, as compared to ST, improved BC of CM when caused by streptococci, specifically *Strep. uberis*. At day 1.5, only 13% of quarters showed CC, increasing significantly towards 60% at day 5, and 99 % at day 14 and at day 21. No significant difference in CC was present between treatment groups. Overall SCC cure was low (22%) and not significantly different between treatment groups, but significantly higher for cases due to enterobacteriacae compared to staphylococci.

In conclusion, ET with cefquinome of CM in cows with a persistent high SCC seems to be only indicated when caused by streptococci, mainly *Streptococcus uberis* but shows no advantage when no information on bacteriological causes of mastitis is available. In our data, absence of CC directly after ST was not related to eventual BC.

Keywords: dairy cow, lactation, extended therapy, clinical mastitis, antimicrobial

INTRODUCTION

Mastitis is a painful disease for dairy cows and among the most costly diseases on dairy farms (Halasa *et al.* 2009). Clinical mastitis (CM) treatment protocols are an important part of mastitis control programs and are generally aimed at maximizing bacteriological cure (BC). The majority of antimicrobial products approved for CM treatment have a dosing regimen of 1-2 days. This dosing scheme is sometimes perceived too short for antibiotics that are frequently used in mastitis treatment. To enhance BC of persistent IMI extending the duration of treatment to 5 or even 8 days has been proposed and shown to be effective for subclinical mastitis caused by streptococci and *Staphylococcus aureus* (Gillespie et al., 2002, Oliver et al., 2004*b*, Deluyker *et al.* 2005, Roy et al., 2009). For CM, extended treatment (ET) has particularly been advocated for *Streptococcus uberis* (Oliver *et al.* 2004*a*, Milne *et al.* 2005, Krömker *et al.* 2010). The reported effects of ET on *Staphylococcus aureus* CM vary ranging from beneficial (Truchetti et al., 2014), only beneficial for β-lactamase negative strains (Jarp *et al.* 1989, Sol *et al.* 2000) to no difference (Pyörälä & Pyörälä, 1998, Swinkels *et al.* 2013*a*). This variability in effect may be due to differences in study design, type of antibiotics used and route of application. Studies on the added value of ET compared to standard treatment (ST) of *Escherichia coli* CM are scarce, likely because intramammary *E. coli* infections are considered transient, self-limiting and thus an additional effect of antibiotic therapy is not expected (Pyörälä & Pyörälä, 1998). However, in trials including more persistent *E. coli* infections, ET has been shown to be more effective than no treatment (Schukken *et al.* 2011).

If cows with CM are perceived to not clinically respond to ST, farmers usually extend treatment, thereby expecting a higher rate of cure. That type of CM cases may be due to clinical flare-ups of persistent subclinical IMI. These persistent IMI have been reported as difficult to cure (Deluyker *et al.* 2005) and to show clinical flare-ups during the course of an IMI (Lam, 1996). It is likely that persistent IMI can be identified through a prolonged elevated SCC before the onset of clinical signs (De Haas *et al*. 2002). Using information on elevated SCC before the onset of clinical symptoms may therefore be a valuable tool in advising dairy farmers to choose extended therapy. However, convincing evidence of a beneficial effect of ET of CM cases in cows with persistent high SCC (**CMPHS**) is lacking. This lack of evidence is particularly true when the causal pathogen is unknown, which in practice usually is the case when a CM treatment is started. Cefquinome is a broad-spectrum β-lactam antibiotic, licensed throughout Europe for the intramammary treatment of CM caused by all major mastitis pathogens. In practice, in the absence of previous identification of the causative pathogen, the aetiology can be diverse and may justify a broad-spectrum antibiotic for first treatment of CM. The objective of this trial is to compare the effect of standard 1.5 day versus extended 5-day cefquinome treatment of CMPHS.

MATERIAL AND METHODS

Animal welfare

Ethical use of animals approval was requested and approved in August 2010 by the Lower-Saxony consumer protection and food safety State Office, under number 10A062.

Study design

This was a non-blinded, systematically allocated and controlled CM trial comparing 2 treatment groups. The study was conducted on 20 dairy farms from August 2010 to August 2012. The efficacy of a standard, 1.5 day, and an extended, 5-day, intramammary cefquinome treatment were compared. Only cows with CM preceded by at least two consecutive monthly elevated SCC > 200,000 cells/ml were included. Primary efficacy criteria were BC and CC, while SCC cure was considered secondarily. The definitions of the efficacy criteria are described below.

Herds

To be able to identify farm effects and to finish the study in a reasonable time frame, 20 dairy farms were selected. These farms were located in the German federal states of Saxony-Anhalt, Lower Saxony and North Rhine-Westphalia. They were all free-stall farms with the ability to demonstrate compliance to the study protocol. For inclusion, cows had to be easily identifiable by ear tag or freeze branding. Farms needed to monthly record individual cow SCC, for at least 12 months before the start of the study. No specific requirements related to Bulk Milk SCC or CM incidence were necessary for inclusion into the study.

Inclusion of cows

First inclusion criterion called only for lactating cows with CM signs in one quarter. Cows with clinical signs in more than one quarter were not included in the study. Of these CM cases, only those immediately preceded by at least 2 consecutive monthly milk recordings after calving, showing high cow SCC (>200,000 cells/ml), described as CMPHS, were included. The first SCC recording for all cows was at or after 3 days post-calving (Barkema et al., 1999). Clinical mastitis was defined as a quarter with changes in the appearance of milk, with or without clinical signs of the affected quarter (swelling, heat, pain) and with or without associated general clinical signs.

Following identification of CM by a trained milker, cows were assessed for exclusion criteria. Cows were excluded if they did not fulfil the SCC criteria, had quarters with significant udder, teat and teat orifice lesions, had had a CM in the immediately preceding month, were treated with other products in addition to the intramammary antibiotic or had concurrent diseases at the time of CM. Additionally, farmers were allowed to exclude cows if they decided for additional parenteral treatment in severe CM cases. Parity, affected quarter location, days in milk at CM occurrence, milk production, cow SCC measured at the two most recent milk recordings before

CM, and relevant clinical data were collated. The first day of treatment constituted initiation of the study for each included cow (d0). Cows were only included once in the study. If ≤ 2 pathogens were cultured in both (duplicate) pre-treatment samples, a case was included in the study. If in one of the duplicate samples > 2 pathogens were cultured and not in the other, the same pathogen found in both samples, were considered the causative mastitis pathogens and the case was included.

Post admission withdrawal

For practical reasons, farmers were allowed some flexibility and had the right to withdraw a cow from the study post admission after consultation with the veterinarian. These cases were carefully documented. In the case of adverse events, the protocol included that the cows were carefully checked for by the milker and monitored by the herd veterinarian. If in both (duplicate) samples > 2 pathogens were cultured, a case was considered contaminated and excluded from the analysis. For further details, see laboratory methods.

Number of cows

The tested hypothesis was that ET resulted in a higher BC rate (65%) than ST (50%). Based on a one sided Chi-square test with type I error α=0.05 and type II error ß=0.20, a total of 175 animals were needed per treatment group. Assuming that approximately 10-15% of cows drop out of the trial post admission, approximately 200 cows were needed per treatment group, in total 400 cows with CM.

Randomisation

After sampling, cows were systematically allocated to each treatment group based on the last ear tag or freeze brand number. Ear tag or freeze brand numbers were consecutively allocated to individual cows at the moment they joined the herd. Cows with odd numbers received ST while cows with even numbers received ET.

Data recording

At the cow level, data were either recorded cow-side onto data capture forms or retrieved onto data forms from on-farm software at the time of treatment. Any disease or concurrent treatments were recorded for a period of 25 days after enrollment.

Milk sampling

Before the start of the study, farms were provided with all sampling materials. One pre- and 2 post-treatment milk samples were taken, according to the CVMP guideline for efficacy studies of intramammary products (CVMP, 2013). Milk sampling of CM cases was performed by the

milkers after specific instructions given by the herd veterinarian. Duplicate quarter samples from the clinically affected quarter were collected aseptically before treatment and sent to the microbiological laboratory at the University of Applied Sciences, Hannover by post. Post treatment quarter milk samples were taken in duplicate at 14 (± 2) and 21 (± 2) days post enrollment, bacteriology and SCC measurement by a trained veterinarian.

Laboratory Methods

Microbiological methods were based on National Mastitis Council recommendations (NMC, 1999) as cited by the German Veterinary Association (GVA, 2009). All milk samples collected were cooled (at or below 8°C), but were never frozen. Samples were kept in the refrigerator or in cooling boxes or bags during transport. The interval between sampling and analysis was < 36 hours for CM samples and < 8 hours for post treatment samples. One hundred µl of milk from each duplicate sample was plated onto an esculin blood agar plate (Oxoid, Germany) and on Chromocult® Coliform Agar (Merck, Germany). Both plates were incubated aerobically at 37 °C and examined after 24 h. The esculin blood agar plates were also examined after 48 h (GVA, 2009). Definitions of culture status were according to GVA (2009) procedures. Bacterial cultures of cow associated pathogens (*Staphylococcus aureus*, *Streptococcus agalactiae*, Streptococci type C, *Trueperella pyogenes*) were considered positive if ≥ 10 cfu/ml were isolated in both (duplicate) samples. Bacterial cultures of environment associated pathogens (*Strep. uberis, E. coli, Klebsiella* spp., other coliforms, *Enterococcus* spp., CNS, *Pseudomonas* spp.) were considered positive only if ≥ 100 cfu/ml were isolated in both (duplicate) samples. Categories of the number of cfu/ml of the isolated pathogens were documented as1: 10-100 cfu/ml; 2: 101-500 cfu/ml or 3: >500 cfu/ml. Of every CM, both (duplicate) samples were cultured. If 2 pathogens were cultured in both duplicate samples, it was considered a mixed infection. In the case 2 distinct pathogens were cultured from one of the duplicate samples, while from the second duplicate sample only one of these pathogens was cultured, the pathogen that was cultured in both samples was identified as the causative pathogen. If > 2 pathogens were cultured in both (duplicate) samples the case was considered contaminated. After isolation of bacterial species, bacterial strain typing was performed using randomly amplified polymorphic DNA analysis (RAPD) as described earlier (Gillespie & Oliver, 2004, Naffa *et al.* 2006, Pacheco *et al.* 1996, Vogel *et al.* 1999). Directly after isolation of bacteria, the samples were stored at -80 °C. At the end of the study, the samples were thawed, the bacterial strains re-cultured and at the same time, the RAPD test was performed on the same agar gel. RAPD was done on one of the bacteriologically positive duplicate pre-treatment samples and on one of the duplicate post treatment samples at day 14 and at day 21. Interpretation of the RAPD was done as described earlier (Munoz *et al.* 2007).

The SCC of quarter foremilk samples were collected in tubes containing boric acid and were cooled in the refrigerator at 4 °C during transport to the laboratory. Immediately after arrival in the laboratory, SCC was determined by flow cytometry with the Somascope Smart (Delta Instruments, The Netherlands).

Treatment

Treatment was performed by the milkers. Two different intramammary treatment regimens with 75 mg cefquinome (Cobactan ® LC, MSD Animal Health) were investigated in this study, either an on label,1.5-day treatment with 3 tubes at 12 hours intervals (ST), or an off-label, 5-day treatment with 6 tubes, 3 tubes at 12 hours interval, followed by 3 tubes at 24 hour interval, 1 each day (ET). These intervals are in compliance with pharmacokinetic characteristics of the intramammary product to maintain appropriate drug concentration.

Clinical evaluation

Before the start of the study, milkers were trained by the veterinarian in evaluation and documentation of clinical signs. Clinical evaluation was done by the milkers at the onset of CM. At day 1.5 (36 hours after the start of treatment) and day 5 (120 hours after the start of treatment) clinical evaluation was also performed by the milkers and at day 14 and 21 by the same herd veterinarian. Clinical evaluation consisted of a classification of severity of disease as Grade 0; normal, Grade 1; mild, only clots in the milk, Grade 2; moderate, symptoms of grade 1 and heat, pain and/or swelling of the udder, rectal temperature <39.5°C and Grade 3; severe, symptoms of grade 2 and depression, anorexia, recumbency, rectal temperature >39.5°C. At least 2 generalized symptoms needed to be present for grade 3 classification.

Definitions

Bacteriological cure was defined as the absence of the bacterial strain isolated pre-treatment, based on both bacteriological culturing and RAPD strain typing, in both (duplicate) post treatment samples at day 14 and 21. The objective of including RAPD strain typing in the definition of BC was to get a more accurate diagnosis. For example, when the same bacterial species was identified in the bacterial culture pre- and post-treatment, but RAPD typing revealed a different strain pre- and post-treatment, the case was defined as a BC. Clinical cure was defined as the absence of any clinical sign of mastitis (grade 0) after treatment and was defined at 4 different time points: at day 1.5, day 5, day 14 and day 21. Somatic cell count cure was defined as a quarter SCC being < 200,000 cells/ml both at day 14 and day 21.

Blinding

By virtue of the differences in treatment regime it was not possible to blind the study personnel or the farmer or herdspersons to product administration. The personnel at the laboratory, culturing milk samples, were unaware of the treatment given.

Statistical Analysis

To test homogeneity of data of the two treatment groups, normally distributed metric data were tested statistically with a Student's t-test. Nominal data, i.e., clinical score, were compared as proportions with a χ^2-test. Although the affected quarter was the unit of observation for BC, only one quarter per cow was included and therefore cow and quarter analysis are identical. In the case of BC, if 2 different pathogens were isolated, each pathogen was analyzed separately and two data lines were included in the analysis for BC. Bacteriological cure, CC and SCC cure were evaluated using mixed model logistic regression analysis where parity, treatment duration (treatment), pathogen group (enterobacteriaceae, streptococci, staphylococci, no growth and other), quarter position (front/hind), cow SCC pre-treatment (measured at the most recent milk recording before CM occurrence), days in milk (DIM) and cfu category (1, 2 or 3) were included as fixed effects and farm was included as random effect. Somatic cell count cure was categorized according to the cut-off value of 200,000 cells/ml as mentioned earlier. For the statistical analysis, SAS, version 9.2 (SAS Institute, Inc., Cary, NC, USA) was used. The full model, including possible confounders and interaction term, was given by:

Logit (BC, CC, SCC) = Lactation + DIM + quarter position + pathogen-group + treatment + pre-treatment cow SCC + cfu category + treatment × pathogen-group + Herd (random) + e

A stepwise-backwards analysis was performed using $p < 0.05$ for inclusion and $p > 0.10$ for exclusion and controlling for potential confounding. Essentially, the interaction term allows us to test the pathogen-group specific effect of ET versus ST.

RESULTS

Farms

The cows came from herds with a wide range of herd sizes (100-1261 cows/farm), 305 d milk production (7840-12202 kg), and most recent BMSCC before the start of the study (164.000-368.000 cells/ml). All farms milked twice daily, used gloves during milking and a single tissue per cow for cleaning teats before milking, applied post-milking teat disinfection and blanket antibiotic dry cow treatment to all cows throughout the study.

Cows

A total of 435 cows met the inclusion criteria. Sixteen cows were excluded from the study due to additional parenteral treatment in severe CM cases. A total of 419 cows were enrolled and sampled pre-treatment for bacteriological examination. No adverse events of treatment were seen. Seven cows were withdrawn post-admission due to missing data. Three cases were withdrawn post-admission due to off-protocol treatment; 2 cases due to additional parenteral treatment and 1 case due to treatment with another intramammary antibiotic. Finally, 409 cows, 203 in the ST and 206 in the ET group, completed the study and were included in the analyses.

Homogeneity of treatment groups

No significant differences were found in parity, DIM, milk production, pre-treatment cow SCC, enrolment quarter SCC, enrollment bacteria count (cfu/ml) and clinical score at the start of treatment between the ET and the ST group (P>0.05, Table 1).

Table 1. Descriptive cow level data of groups of standard (n=203), 1.5 day, and extended (n=206), 5 day intramammary cefquinome treatment of clinical mastitis in cows with persistent high SCC from 20 different herds in Germany. Most parameters were compared with a Student t-test and are presented as the mean with the standard deviation in parentheses.

Cow level	Standard	Extended	P-value
Parity	3.4 (1.8)	3.5 (1.8)	0.29
Days in milk (days) †	196 (128)	182 (98)	0.11
Milk production (kg/day) ‡	33.8 (9.9)	33.7(10.3)	0.46
Pre-treatment cow SCC(log) §	5.87 (0.51)	5.87(0.45)	0.50
Enrolment quarter SCC(log)	6.79 (0.37)	6.72 0.52)	0.26
Bacteria count (cfu category) ¶	2.53 (0.72)	2.55(0.70)	0.47
Median clinical score (min-max) ††	2 (1-3)	2 (1-3)	0.60

† Days in milk at the day of clinical mastitis occurrence
‡ Milk production at the last milk recording before clinical mastitis
§ Cow somatic cell count measured at the last milk recording before clinical mastitis
¶ Bacterial count of the clinical mastitis causing pathogen ; 1: 10-100 cfu/ml; 2: 101-500 cfu/ml or 3: >500 cfu/ml.
†† *χ-^{2}test (likelihood ratio statistic) for proportions*

Isolated bacterial species

The most frequent isolated pathogens were *Strep. uberis* (n=115; 28%), *E. coli* (n=54; 13%), other coliforms (n=34; 8%), CNS, including CNS from mixed infections (n=34; 8%) and *Staph. aureus*, (n=32; 8%) (Table 2). Sixty samples showed no growth (15%). Four quarters showed mixed infections and there were no quarters with duplicate contaminated samples.

Bacteriological cure

The overall BC of all CMPHS cases at day 21, was 78% (135/172) after ET and 72% (127/177) after ST (Table 3). At the pathogen level, streptococci, mainly *Strep. uberis*, showed a better BC after ET (83%) than after ST (65%), while BC of *Staph. aureus* was lower after ET (40%) than after ST (64%) (Table 3). Least square means of BC after ST was 72% and was 79% after ET. In the final model, BC after ET was significantly different from ST for streptococci, mainly *Strep. uberis*, but not for other pathogens (Table 4). Cases associated with streptococci were 3 times more likely to cure bacteriologically after ET compared to ST (P = 0.04). At the bacterial group level, BC of staphylococci was significantly lower than BC of 'other pathogens'. There was

no significant difference between the BC of streptococci (P = 0.08) and of *enterobacteriaceae* (P=0.98) versus 'other pathogens'. The random farm effect was not significant (P=0.19) but was kept in the model as a design variable.

Table 2. Bacteriological culture results of milk samples of 409 clinical mastitis cases in cows with persistent high SCC from 20 different herds in Germany. Results are presented in total and in groups of cows with standard, 1.5 day, and extended, 5 day intramammary cefquiniome treatment.

Bacteria	Standard	Extended	Total	%
E. coli	27	27	54	13%
Other Coliforms †	19	15	34	8%
Klebsiella spp.	2	3	5	1%
Serratia marcescens		1	1	0.2%
Enterobacteriaceae ‡	48	46	94	
Strep. uberis	56	59	115	28%
Strep. dysgalactiae	10	7	17	4%
Strep. canis	1	2	3	1%
Strep. agalactiae		1	1	0.2%
Streptococci §	67	69	136	
Staph. aureus	22	10	32	8%
CNS	16	14	30	7%
Staphylococci ¶	38	24	62	
No growth	26	34	60	15%
Enterococci	8	6	14	3%
Coryneform	3	9	12	3%
Bacillus spp.	2	6	8	2%
Other	11	12	23	6%
Total	203	206	409	100%

† Coliforms other than *E. coli*, *Klebsiella spp. and Serratia marcescens*
‡ *E. coli*, *Klebsiella spp., Serratia mercescens* and other Coliforms
§ *Strep. uberis, Strep. dysgalactiae, Strep. agalactiae, Strep. Canis*
¶ *Staph. aureus* and CNS

Clinical cure

The evolution of CM signs during the trial is presented in Figure 1. At enrolment, 77% (314/409), of CM cases were mild (grade 1), 21% (85/409) moderate (grade 2) and 2.5% (10/409) severe (grade 3). At 1.5 day after enrolment, 13% (54/409) was clinically cured, 70% (288/409) had mild, 15% (61/409) moderate and 0.7% (3/409) severe signs. At 5 days after enrolment clinical signs had significantly improved (P < 0.01) and 62% (126/203) of the cows in the ST group and 58% (120/206) in the ET group were clinically cured. At 14 and 21 days after treatment almost 100% of CM cases were clinically cured. In the final model for CC, ET was not significantly different from ST (P=0.21). Cows affected with CM within the first 100 DIM had a significantly lower CC compared to cows affected after 200 DIM (P=0.02).

Table 3. Descriptive data of bacteriological cure (%) by bacterial group after standard (n=203), 1.5 day, and extended (n=206), 5 day, cefquinome treatment of 409 clinical mastitis cases in cows with persistent high SCC from 20 different herds in Germany. Numbers of cures divided by the number of cases are presented in parenthesis.

	Bacteriological cure	
Bacterial category	**Standard**	**Extended**
Enterobacteriaceae[†]	81 (39/48)	83 (38/46)
E. coli	93 (25/27)	85 (23/27)
Staphylococci[‡]	65 (26/40)	58 (15/26)
Staph. aureus	64 (14/22)	40 (4/10)
Streptococci[§]	65 (50/77)	83 (63/76)
Strep. uberis	64 (36/56)	81 (48/59)
Other[¶]	79 (26/33)	83 (34/41)
Total	72 (127/177)	78 (135/172)

[†] *E. coli*, *Klebsiella spp.* and other Coliforms
[‡] *Staph. aureus* and CNS
[§] Enterococci, *Strep. uberis, Strep. dysgalactiae, Strep. agalactiae, Strep. canis*
[¶] Coryneforms, *Bacillus spp.*, *T. pyogenes*, *Pseudomonas spp.*, Yeasts, *Prototheca spp.*

Table 4. Final mixed logistic regression model for bacteriological cure of clinical mastitis in cows with persistent high SCC (n=409) from 20 different herds in Germany. Cows were either treated with a 1.5 day standard, or a 5 day extended, intramammary cefquinome treatment. Herd was included as a random effect.

Effect	**Estimate**	**SE**	**DF**	**t-value**	**P-value**	**OR**	**95% CI**
Intercept	1.66	0.51	19	3.25	0.004		
Extended treatment vs. Standard treatment	-0.20	0.36	312	-0.57	0.57	0.82	0.40 - 1.66
Enterobacteriaceae	-0.01	0.52	312	-0.03	0.98	0.99	0.36 - 2.7
Streptococci	-0.99	0.56	312	-1.78	0.08	0.37	0.12 - 1.1
Staphylococci vs. Other	-1.12	0.52	312	-2.16	0.03	0.33	0.12 - 0.91
Extended treatment x Streps vs. Standard treatment x Streps	1.12	0.54	312	2.07	0.04	3.06	1.06 - 8.87

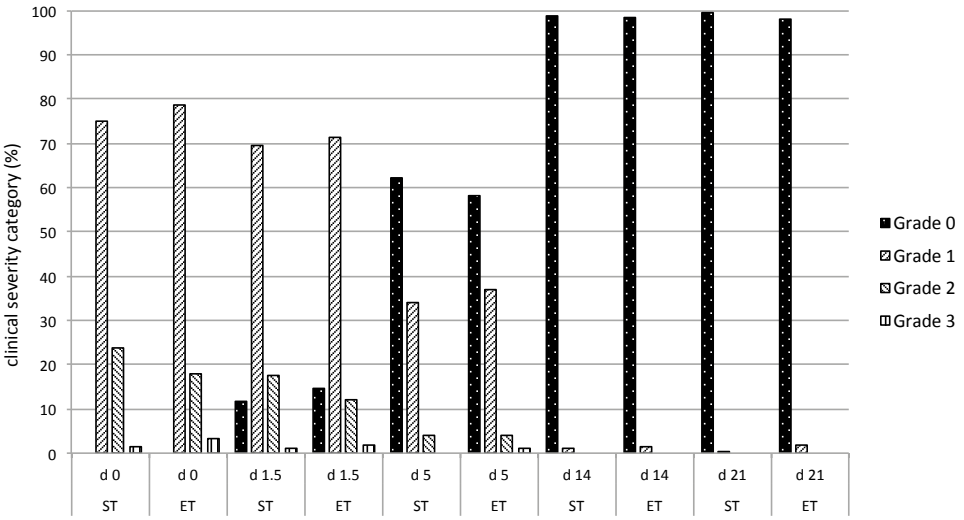

Figure 1. Percentage of clinical mastitis cases in severity categories Grade 0, 1, 2, and 3† at the time of first treatment (day 0) and at day (d) 1.5, 5, 14 and 21 after treatment of cows with persistent high SCC from 20 different herds in Germany. Cows were either treated with a 1.5 day standard (ST, n=203), or a 5 day extended (ET, n=206), intramammary cefquinome treatment.

† Grade 0: healthy, no clinical signs of mastitis, Grade 1: mild, only clots in the milk, Grade 2: moderate; heat, pain and/or swelling of the udder, rectal temperature <39.5°C, Grade 3; severe, symptoms of grade 2 and depression, anorexia, recumbency, rectal temperature >39.5°C. At least 2 generalized symptoms needed to be present for grade 3 classification

Somatic cell count cure

The development and cure of SCC are shown in Table 5. Overall SCC cure rate was 22%, being 19% after ST and 25% after ET. The SCC cure of the different pathogens or pathogen groups was; enterobacteriaceae (30%), culture negative (25%), staphylococci (17%), *Strep. uberis* (17%) and others (22%). Least square means of SCC cure was 19 % after ST and 25% after ET. In the final model, SCC cure after ET was not significantly different from ST (P=0.15), nor was the interaction term of treatment x pathogen (Table 6). SCC cure was significantly higher only for enterobacteriaceae (P = 0.047) compared to staphylococci. Random farm effect was not significant (P = 0.41) but was kept in the model as a design variable.

Table 5. Development and cure of mean log quarter somatic cell count (SCC) (cells / ml), after treatment of clinical mastitis cases of cows with persistent high SCC, from 20 different farms in Germany, with intramammary cefquinome, either standard (n=203), 1.5 days, or extended (n=206), 5 days.

	Pre-treatment†	day 0	day 14	day 21	SCC cure‡
Standard	5.87 ± 0.51	6.79 ± 0.37	5.81 ± 0.82	5.80 ± 0.85	19%
Extended	5.87 ± 0.45	6.72 ± 0.52	5.70 ± 0.83	5.72 ± 0.93	25%

In addition to the mean log quarter SCC, standard error of the mean (±) is indicated for pre-treatment (cow level), and of clinical mastitis cases at day 0, day 14 and day 21 (quarter level) in the standard and extended treatment group. Differences between treatment groups were not significant ($P > 0.05$).

† Cow SCC recorded at the most recent milk recording before the occurrence of the clinical case.

‡ Quarter SCC < 200,000 cells/ml at both day 14 and 21 after enrolment.

Table 6. Final mixed logistic regression model for somatic cell count cure of clinical mastitis in cows with persistent high SCC (n=409) from 20 different herds in Germany. Cows were either treated with a 1.5 day standard, or a 5 day extended, intramammary cefquinome treatment. Herd was included as a random effect

Effect	Estimate	SE	DF	t-value	P-value	OR	95% CI
Intercept	-1.86	0.37	19	-4.95	<0.0001		
Extended treatment vs. Standard treatment	0.36	0.25	384	1.44	0.15	1.43	0.88 - 2.34
Enterobacteriaceae	0.84	0.42	384	1.99	0.047	2.32	1.01 - 5.29
No growth	0.55	0.47	384	1.18	0.24	1.74	0.69 - 4.35
Other	0.40	0.52	384	0.77	0.44	1.50	0.53 - 4.19
Streptococci vs. Staphylococci	0.11	0.41	384	0.27	0.79	1.1	0.50 - 2.51

DISCUSSION

In practice, farmers frequently extend CM treatment if clinical signs persist after completion of ST, expecting better BC and CC. Our study showed that ET with cefquinome of CMPHS cases improved BC when streptococci were cultured, but not for other pathogens (P=0.57, Table 4). The non-significant results of the comparison of ST and ET irrespective of the underlying pathogen is in contrast with a recent study (Truchetti et al, 2014), that showed an overall difference in BC after extended CM treatment compared to standard treatment. Although the latter study also included mild to moderate CM as was mainly the case in our study, the duration of extended treatment was 8 days compared to 5 days in our study. This suggests further prolongation of treatment duration beyond 5 days may improve BC compared to standard treatment in mild to moderate CM. We were interested in the effect of CMPHS treatment without prior knowledge of the underlying pathogen, because that reflects the situation in daily practice. Power calculations were therefore

based on finding effects at the overall treatment level rather than at the underlying pathogen level. When analyzing the data at the pathogen level, lack of power may be an important cause of non-significant results. A minimum of 175 cases of each pathogen would have been required to show an increase of 15% cure assuming a 50% cure with ST. Another possible reason for the lack of significance is that the ST group showed a higher cure rate than expected (72%), leaving limited room for improvement.

We found BC after ET of streptococcal CMPHS, mainly due to *Strep. uberis*, to be significantly higher (P = 0.04) compared to ST. This is line with previous research (Oliver *et al.* 2004*a*, Milne *et al.* 2005, Krömker *et al.* 2010) suggesting ET may have added value for treatment of CM caused by *Strep. uberis*. It is in contrast, however, with findings of Truchetti *et al*. (2014) who could not find an improved BC after extended treatment of streptococcal CM, which may have been caused by lack of a statistical power. For pathogens such as *E. coli*, we did not expect an additional effect of ET because these are considered to generally be transient, self-limiting infections. This expectation was confirmed by the very high BC rate (93%) of *E. coli* after ST. For staphylococcal CM, we expected a positive effect of extended treatment of *Staph. aureus* CM, recently shown by Truchetti *et al.* (2014) and previously by Jarp *et al.* (1989) or Sol *et al.* (2000) for *Staph. aureus* CM caused by β-lactamase negative strains. We could, however, not prove a beneficial effect of ET compared to ST in *Staph. aureus* CMPHS, putting our findings in line with previous work in our group (Swinkels *et al.* 2013*a*), indicating that CMPHS cases probably are different from CM cases in general. We even found a lower BC after ET (40%) of *Staph. aureus* CMPHS as compared to ST (64%). This may be a spurious finding, due to the low number of *Staph. aureus* cases in the ET group (n=10). Another cause for the discrepancy between the effect of ET on *Strep. uberis* and *Staph. aureus* CMPHS may be the capabilities of *Staph. aureus* to invade mammary epithelial cells (Hensen *et al.* 2000, Kerro Dego *et al.* 2002) or to survive in neutrophils (Mullarky *et al.* 2001). Thus, *Strep. uberis* IMI may be more sensitive to extended exposure of antibiotics than *Staph. aureus*. The ability of *Staph. aureus* to protect itself from antibiotics could also have contributed to the significantly lower BC after both treatments of staphylococci as compared to other pathogens (P=0.03, Table 4).

Strain typing is usually not performed in treatment efficacy trials because it is laborious and costly. Strain typing, however, does increase reliability of results, because the identification of a different bacterial strain after treatment, does provide additional information on BC. Although misclassification may occur, sensitivity and specificity of RAPD testing in streptococcal species, the most frequently isolated pathogens in our study, is relatively high (90% and 92% respectively; Gillespie *et al.* 1997), suggesting reliable results. Based on the RAPD results, overall BC increased by approximately 4 - 5% in the ST and by 3% in the ET group compared to the BC without RAPD (data not shown), indicating that antibiotic treatment may lead to a higher cure rate than usually reported (Schukken *et al.* 2011).

It has been suggested that correlations between SCC patterns and CM occurrence can be used in mastitis control (De Haas *et al.* 2002; 2004). We could not prove that ET of CMPHS cases increased BC as compared to ST. For this reason, and because ET cannot easily be justified economically (Steeneveld *et al.* 2011), routine cefquinome ET protocols for CMPHS cases is not

recommended. An exception may be the use of ET for cows known to have a streptococcal IMI or on farms where mastitis is predominantly caused by streptococci.

We expected to predominantly isolate pathogens known to cause persistent IMI, such as *Staph. aureus* and *Strep. uberis*. Interestingly, after *Strep. uberis*, *E. coli* was the most frequently isolated pathogen. This suggests that clinical *E. coli* cases either occurred as a mixed infection or as an opportunistic infection in other quarters than those with an already elevated SCC. Other possibilities are that *E. coli* infections occurred in a quarter that cured from the previous infection between the last DHI test and the CM caused by *E. coli*, or that an *E. coli* infection itself causes elevated SCC, confirming that *E. coli* also causes persistent IMI, as was previously reported (Bradley & Green, 2001, Döpfer *et al.* 1999, Dogan *et al*. 2006).

In practice, farmers judge treatment success on the disappearance of all clinical signs. At day 1.5, 85% of CM cases still had clinical signs, likely perceived as 'treatment failure', encouraging ET. At day 5, 14 and 21, the percentage of cows with clinical signs had decreased significantly, irrespective of treatment protocol, suggesting CC simply needs more time instead of more antibiotic exposure time. These findings suggest that after cefquinome treatment, removal of the visible debris of inflammation takes more time than the duration of treatment as indicated on the label. The apparent gap between overall CC (99%) both at day 14 and 21 and BC (75%) at the same time points may indicate that, after initial cure, re-infection occurs with the same bacterial strain, or that a substantial part of CMPHS cases turn back to a subclinical status, with possible clinical flare-ups later on. Cows not cured from CM cases caused by contagious pathogens could therefore be at risk for spreading the infection to healthy herd mates. These findings emphasize CM treatment protocols are only a limited part of mastitis control programs and can benefit from being combined with other mastitis control measures, such as teat dipping, milking hygiene and proper milking machine maintenance, to reduce transmission rate and have an optimal effect (Barlow *et al.* 2009, Halasa, 2012).

Somatic cell count cure is important to the dairy farmer because SCC, rather than BC is routinely used by the milk processing industry as a measure of milk quality. Extended treatment did not result in significant further reduction of SCC compared to ST. The overall reduction of quarter log SCC after treatment was limited (approximately 6.7 to 5.8 log units) and the overall SCC cure was low (22%), especially when compared to BC (76%) and CC (99%). This is in line with findings of others (St. Rose *et al.* 2003) and suggests that short term benefits of treatment of CMPHS cases are the elimination of bacteria and clinical signs rather than reduction of SCC. The SCC cure for both treatments, however, was significantly higher for *enterobacteriaceae* (mainly *E. coli* cases), than for staphylococci ($P = 0.047$). This is line with De Haas *et al.* (2004) who showed that *E. coli* CM has only a short peak in SCC, whereas *Staph. aureus* CM showed a long term increased SCC.

Due to practical limitations of a field trial in commercial herds, farmers were allowed to exclude cows fulfilling all the inclusion criteria, from entering the study. Sixteen clinically severe CM cases (3.6% of all CM cases) that met the inclusion criteria, were left out of the study, because milkers decided to treat them with an additional parenteral treatment. In 10 (2.5%) CM cases, that were

judged as severe (grade 3) by the milkers, 4 (1.0%) in the ST group and 6 (1.5%) in the ET group, the milkers decided to treat only intramammary. These cases were included in the study. Exclusion of most severe cases has contributed to a considerably lower percentage of severe cases (2.5%) in our study than the 10% and 7% previously reported in Germany (Krömker *et al.* 2013) and in the UK (Swinkels *et al.* 2013*b*). Thus, our conclusions mainly relate to mild and moderate CMPHS cases and reflect the field situation where severe cases are often treated parenterally. The added value of parenteral treatment on BC, CC and SCC cure was not evaluated in our study and would need specific attention.

Another practical limitation was that milkers could not be blinded. For CC at day 1.5 and 5 it was the same milker, and at day 14 and 21 the same veterinarian who performed the clinical scoring. The milker was aware of the treatment given but the veterinarian was not. Because the milkers knew the treatment protocol of cows at clinical scoring, we cannot fully exclude bias at that point. However, we consider it unlikely this affected the outcome of the study because results of milkers did not differ from those of the veterinarians, showing no difference in CC between treatment groups. Additionally, CC results were in the same direction as BC and SCC results.

In conclusion, intramammary cefquinome ET as compared to ST in cows with CMPHS improved BC when caused by streptococci, specifically *Strep. uberis*. This could not be shown for other pathogens, suggesting ET with cefquinome of CMPHS cases may be appropriate only to farms with predominantly streptococci, such as *Strep. uberis*, as the causative mastitis pathogen and shows no advantage when no information on bacteriological causes of mastitis is available. Specifically farms with on-farm culture programs may benefit from these findings (Lago et al., 2011*a, b*).

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CHAPTER 7

Social influences on the duration of antibiotic treatment of clinical mastitis in dairy cows

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ABSTRACT

Clinical mastitis of dairy cows is a visible inflammation of the udder, usually caused by bacteria and treated with antibiotics. Although pressure to reduce antibiotic usage in livestock is mounting in the EU, feedback from the field suggests clinical mastitis treatment is frequently repeated after initial label treatment, thereby extending treatment duration. The aim of this study was to gain insight into the social factors influencing farmers' decision making on the duration of antibiotic treatment of clinical mastitis. A total of 38 dairy farmers in The Netherlands (n = 17) and Germany (n = 21) were interviewed in a qualitative semi-structured way. Extended treatment was any treatment longer than label directions. Of the 38 farms, 30 reported routine and 7 occasional extended antibiotic treatment. The interviewed dairy farmers are sensitive towards social norms of other farmers and towards recognition for good stockmanship. Extended treatment is perceived as to be part of the social norm of 'being a good farmer'. The farmers' perception is that mastitis is not treated 'thoroughly', if clinical symptoms are still visible at the time of cessation of treatment, because it may persist or recur. As a result, treatment is frequently extended by repeating the initial label treatment. Farmers, specifically more 'cow-oriented' farmers, expressed insecurity on how to treat mastitis effectively. This insecurity makes them more sensitive to comply to other farmers injunctive ('what ought to be') and descriptive ('what is done') norms, and to the veterinarians informational norm that extended treatment is better, resulting in an approved social norm. Social approval reduces insecurity of being perceived as a poor farmer and thus, extended treatment is emotionally rewarded. This social reward apparently outweighs higher costs of more waste milk and more antibiotic usage.

Perceived positive reference groups with whom the farmer identifies and regularly communicates face to face, such as other farmers, veterinarians, and other farm advisors, confirm the farmers' judgment on extending treatment and influence them towards socially accepted behavior. Society was the most negative reference group. The emotional gap between farmers and society is large and probably difficult to overcome. Legislation may reduce antibiotic usage, if doable and controllable. Evidence based information on treatment efficacy or practical cow-side criteria to decide on cure, may be able to change social norms of 'thorough' treatment, especially when communicated by a positive reference group such as veterinarians. Because prudent antibiotic use is not improved by subjective norms on duration of antibiotic treatment, more research is needed, particularly on optimal duration of antibiotic treatment of specific pathogens, as related to cure and recurrence of clinical mastitis.

Keywords: dairy cow, clinical mastitis, antibiotic treatment, social influence

INTRODUCTION

Mastitis is a painful inflammation of the udder of dairy cows, usually caused by bacteria. On dairy farms, antibiotics are mainly used to treat clinical mastitis cases and when cows are dried off. Recently, antibiotic use in livestock, including dairy, raised national political concern in The Netherlands. In Germany, the discussion to restrict the use of antibiotics in livestock is currently ongoing, whereas in The Netherlands quantitative goals to reduce antibiotic use in livestock already have been set. In both countries, preventive use of antibiotics has been forbidden. In The Netherlands, this has resulted in the introduction of selective dry cow treatment, only allowing antibiotics to be used at drying off in cows with intramammary infections (Scherpenzeel et al., 2014). Apart from dry cow treatment, further antibiotic reduction in the dairy industry can potentially be realized in changing treatment dosage or duration of clinical mastitis. Feedback from the field, however, suggests clinical mastitis treatment protocols are frequently repeated after an initial on label treatment, thereby extending treatment duration. However, although some studies show a beneficial bacteriological effect of extended treatment of clinical mastitis (Sol et al., 2000, Oliver et al., 2004, Krömker et al., 2010, Truchetti et al., 2014), results are conflicting. Some studies did not find a favorable bacteriological effect of extended treatment of clinical *Staphylococcus aureus* mastitis (Swinkels et al., 2013a), while others did (Truchetti et al., 2014) or showed pathogen specific bacteriological effects in streptococci (Swinkels et al., 2014), or only an impact on clinical signs (Swinkels et al., 2013a,b, McDougall et al., 2014). There seems to be an effect of the type of antibiotic used, the pathogen involved, the duration of infection and possible other factors. The limited scientific data on the added value of extended treatment suggests that extended treatment may not always be necessary and asks for evidence based decisions. Because of the additional associated costs and the mounting pressure to reduce antibiotic use, it seems relevant to explore why so many farmers extend mastitis treatment. Understanding what influences decision making on the duration of treatment, requires insight into farmers' perceptions towards treatment, specifically towards the duration of mastitis treatment.

Behavior, such as extended treatment, is shaped by social interactions between different people (Leeuwis and van den Ban, 2004). Thus, a farmers' decision to extend treatment is not taken in isolation but is influenced by others. It has been reported before that farmers are influenced by both other farmers (Friedman et al., 2007) as well as by their veterinarian (Jansen et al., 2010). However, little is known about *how* farmers are influenced and about the role of other actors within their direct social circle. The objective of studying social influence is to try and understand and explain how the thoughts, feelings and behavior of individuals are influenced by the actual, imagined or implied presence of others (Turner, 1991).

The presence of social norms and social uniformities among the members of a social group arise from their interaction and relationships. Such social norms express social values (different subjective aspects on 'what is believed the majority does or feels') as well as normative judgments (psychological commitments to 'what ought to be' reflecting the consequences of not complying to the rules of the community) (Hechter and Opp, 2001, Bicchieri and Muldoon, 2014). Violation of the norm will result in social sanctions. The theory of social conformity includes that

if a group of people agrees and shares an attitude, that attitude has (subjective) validity (Cialdini and Goldstein, 2004). Group pressure makes such attitudes and beliefs stronger (Festinger, 1950). Theories of social comparison processes imply that people have a need to compare to others to evaluate their opinions and abilities (Cialdini and Goldstein, 2004). Collective wisdom tends to serve the individual and the group as well. Also, people prefer to compare to more similar others. The more similar the others are, the higher their social influence is and the higher the tendency is to reduce differences (Festinger, 1954). Social influence theory distinguishes normative influence when trying to conform to positive expectations of others and informational influence when trying to get information about objective reality (Deutsch and Gerard, 1955). In normative influence theory, injunctive norms ('what ought to be') and descriptive norms ('what is actually done') both play a role. The essential difference between the two is that injunctive norms involve social sanctions for noncompliance with the norm, while descriptive norms do not (Lapinski and Rimal, 2005).

Qualitative research on perceptions of farmers, tries to describe, interpret, and understand their experiences and choices through personal interviews. This qualitative research enables us to catch the entire spectrum of the farmers' social environment that influences their behavior. Qualitative research allows us to study *how* social influences are created and given meaning (Denzin and Lincoln, 2000). Understanding social influence seems to be a prerequisite for effectively influencing behavior, and may be hard to detect if only pre-written quantitative surveys are used. Qualitative research via interviews, on perceptions of farmers towards treatment of clinical mastitis from the perspective of social influence can provide helpful insights into the reasons why farmers extend treatment and thus can contribute to our knowledge on how to influence behavior towards reduced antibiotic use on dairy farms.

The aim of this study is to gain insight into the social influence on decision making on the duration of antibiotic treatment of clinical mastitis of dairy cows in The Netherlands and in Germany, by qualitative research, using personal interviews.

MATERIAL AND METHODS

Selection and description of farms

The target population of this study was non-organic dairy farms not involved in milk processing (only primary production), with a herd size of at least 50 cows in the Netherlands and Germany (North Rhine-Westphalia and Lower Saxony) and a perspective of continuity, expressing the intention to stay in business for at least 5 more years. A census of dairy farmers of The Netherlands and Germany was obtained from the Dutch national respectively the German regional database. Sixty farms were randomly selected from databases. Seventy-two farms were contacted by telephone or by a personal visit to explain the purpose of the study (The Netherlands: 34 farms; Germany: 38 farms). Of the contacted farms 50%, respectively 45% agreed to participate, leading to 17 farms from The Netherlands and 21 farms from Germany involved in this study. This number of farms was considered sufficient, based on earlier reports that after approximately 12 (Guest

et al., 2006), or 20 (Green and Thorogood, 2009) interviews, responses would start to repeat themselves. Average herd size of the Dutch and the German herds was 89, respectively 88 dairy cows, with an average production of 8,930, respectively 9,150 Kg of milk per lactation, which was somewhat higher than the national averages.

Data collection and description

On-farm interviews were carried out between August and December 2013. Interviews were qualitative and semi-structured, and mostly consisted of open-ended questions. We used the same topics to be discussed in each interview in both countries to make sure the information collected would be comparable. Before starting the visits to the selected farms, the interview format was tested to assure questions were clear and to train the interviewers (Baxter and Babbie, 2004). This validation led to slight adaptations of the questionnaire (Table 1). On average, each interview was completed in about 1 hour. Primarily, the decision makers on antibiotic use were interviewed. Sometimes other people, such as the farmers' wife, parent(s) or farm employees were also present during the interviews and gave parts of answers to the questions. Where useful, this information was included in the results.

Table 1. Themes discussed during the semi-structured interviews of Dutch (n = 17) and German (n = 21) dairy farmers

Section	Criteria	Main question
1. Diagnosis of mastitis	Small flakes in milk, big flakes, SCC > 200.000 cells/ml, diseased cow, swollen quarter, CMT-test.	How do you know a cow has mastitis?
2. How mastitis is treated	Intramammary, parenteral, topical, antibiotics or not.	If a cow has mastitis, can you describe how you treat her?
3. Duration of treatment	Number of days, milkings, intramammary tubes. Extended treatment routine, occasional or never. Clinical aspects; recurrent mastitis, presence of flakes, historic SCC level.	When do you stop treatment?
4. Changes in treatment protocol	Triggers, reasons for change, compliance.	Did you always use your current treatment protocol?
5. Social groups, interaction and positive reference	Awareness of what others say or do, how they know, positive references. How, when, effects.	When and with whom do you talk about animal health and mastitis?
6. Social groups, negative reference groups	With whom they disagree or avoid talking about mastitis or antibiotic use. How, when, effects.	With whom do you prefer not to talk about animal health issues, mastitis or antibiotics?
7. Antibiotic use policies	Feelings, attitude, effects.	What is your view on the way civilians and politicians look at the use of antibiotics by the food animal industry?
8. Satisfaction with current treatments	Frustrations, ways forward.	Are you happy with your current treatment protocol?
9. General farm related data	BMSCC, 305-day milkproduction, age.	What is your average BMSCC?
10. What the farmer would like to share	None.	Is there anything else you wish to share?

Table 2. Descriptive data of 21 German farmers, their farm and mastitis treatment characteristics as indicated by the farmer at the time of the interview

Farmer ID	Number of cows	305d milk production (kg)	BMSCC[1] cells/ml	Farmer age[2] (years)	Milking system[3]	Antibiotic treatment duration (days)[4]	Extended antibiotic treatment[5]	Main reason extended antibiotic treatment[6]	Homeo-pathic treatment[7]
DE1	80	7,500	210,000	36	T	3	±	1	+
DE2	110	8,500	275,000	36	T	5-7	+	1,2	-
DE3	110	8,500	125,000	39	T	3-4	+	1	-
DE4	62	8,000	350,000	58	T	2-5	+	1	-
DE5	60	11,750	120,000	41	A	4-5	+	1	-
DE6	88	8,850	117,000	40	T	2.5-5	+	1	-
DE7	120	8,650	150,000	U	T	> 3	+	1	-
DE8	60	10,000	150,000	54	T	3	±	1	+
DE9	120	9,600	120,000	38	T	4	+	1	-
DE10	75	9,900	300,000	U	T	3	+	1	-
DE11	99	9,000	110,000	37	T	3-5	+	1	-
DE12	85	8,000	145,000	50	T	> 4	+	1	-
DE13	80	11,000	100,000	65	T	3-4	±	1	+
DE14	74	9000	180,000	46	A	1-3	±	1	-
DE15	120	9500	225,000	37	T	2-5	±	1	-
DE16	102	11,500	175,000	29	T	3-4	+	1	-
DE17	89	9,200	250,000	U	T	3-5	+	3	-
DE18	80	9,500	300,000	47	T	3	+	1	-
DE19	62	7,750	280,000	48	T	3-4	±	1	+
DE20	68	7,500	240,000	62	T	2-3	±	1	-
DE21	100	9,000	250,000	33	T + A	4-5	+	3	-

[1] BMSCC = Bulk milk somatic cell count
[2] Farmers age; U is Unknown
[3] A is automatic milking system, T is a traditional milking system; any non-automatic milking machine system
[4] Number of days indicated as the most frequently used duration of treatment of clinical mastitis
[5] Extendted antibiotic treatment is treatment length longer than label claim: + applied on the majority of clinical mastitis cases, ± applied occasionally.
[6] N.A. is not applicable because homeopathic treatment is usually applied, 1; insufficient cure of clinical symptoms, 2; fear that mastitis returns, 3; standard protocol
[7] + = standard therapy, - = never used

Due to societal concerns, the use of antibiotics is a sensitive topic in both countries. This was taken into consideration when planning the interviews. We tried to create a natural and safe environment in which the farmers would feel free to speak openly. Two native-speaking interviewers, one in each country, performed the interviews to overcome possible language problems or cultural differences. The interviewers, both in The Netherlands and Germany, were students, who did not position themselves as an expert. It was clearly expressed that there were no right or wrong answers and that the interviewers were simply interested in the way the farmer was treating mastitis.

Table 3. Descriptive data of 17 Dutch dairy farmers, their farm and mastitis treatment characteristics as indicated by the farmer at the time of the interview

Farmer ID	Number of cows	305d milk production (kg)	BMSCC[1] cells/ml	Farmer age (years)	Milking system[2]	Antibiotic treatment duration (days)[3]	Extended antibiotic treatment[4]	Main reason extended antibiotic treatment[5]	Homeo-pathic treatment[6]
NL1	100	8,000	200,000	47	T	> 1.5	+	1	-
NL2	110	9,000	150,000	44	T	> 1.5	+	1,2	-
NL3	115	9,500	200,000	64	A	3	+	1	-
NL4	85	9,000	140,000	49	T	3	+	1,2	-
NL5	85	9,600	125,000	47	T	2-4	+	1	±
NL6	60	8,000	275,000	52	T	3	+	3	-
NL7	90	8,700	100,000	44	T	2	+	1	-
NL8	65	9,171	180,000	48	A	3	+	3	-
NL9	58	8,100	200,000	50	T	3-5	+	2	-
NL10	80	8,600	140,000	48	T	4	+	2	-
NL11	150	10,500	180,000	51	A	2	-	N.A.	-
NL12	55	10,000	100,000	40	T	≥3	+	1,2	-
NL13	85	8,500	180,000	54	T	3-4	+	1,2	-
NL14	100	8,000	175,000	36	T	> 3	+	1,2	-
NL15	70	9,500	100,000	43	A	4-5	+	1,2	-
NL16	130	8,500	190,000	43	T	≥ 3	+	1	-
NL17	70	9,200	130,000	62	T	≥ 3	+	1	-

[1] BMSCC = Bulk milk somatic cell count
[2] A is an automatic milking system. T is a traditional milking system; any non-automatic milking machine system
[3] Number of days indicated as the most frequently used duration of treatment of clinical mastitis
[4] Extended antibiotic treatment is treatment length longer than label claim: + applied on the majority of clinical mastitis cases, - not applied
[5] N.A. = not applicable because repeated treatment was never used, 1; cure of clinical symptoms, 2; fear that mastitis returns, 3; standard protocol
[6] ± applied occasionally, - never used

Duration of treatment

The treatment duration as reported by each farmer is presented as the average number of treatment days per farm in Table 2 and 3. Whether a treatment was considered extended, was based on a comparison of the reported average duration of treatment to the label recommendations. Label treatment duration was 1.5-2 days for the most frequently sold antibiotics in both countries, covering > 90% of total product sales (The Netherlands: FIDIN, 2013; and Germany: VETIDATA, 2014) (Table 4). The interviews showed whether extended treatment was routinely (standard protocol for all cases) or occasionally (only in exceptional cases) applied (Table 2 and 3).

Table 4. Top 8 most frequently sold products registered for the intramammary treatment of clinical mastitis, their approved number of days of treatment of clinical mastitis and their market share (%). In total these products represent 91% of the market in Germany[1] and 92% of the market in The Netherlands[2]

Germany			The Netherlands		
Product	**Market share**	**Treatment duration**	**Product**	**Market share**	**Treatment duration**
1	33	1.5	1	46	1.5
2	15	2	2	31	2
3	15	2	3	15	1.5
4	12	2	4	N.A.[4]	1.5
5	6	1.5	5	N.A.	2
6	6	1-4	6	N.A.	2
7	2	2	7	N.A.	3[3]
8	2	3	8	N.A.	2

[1] VETIDATA, February 2014
[2] FIDIN, 2013
[3] maximum duration of treatment is 3 days
[4] N.A. = Not available

Data Analysis

All interviews were recorded on tape and fully transcribed. The data from the transcribed interviews were ordered and analyzed to identify common patterns and social groups influencing the farmers' decision. Where applicable, results were quantified to get an overview of the results in both countries. Data analysis was based on the grounded theory analysis (Strauss and Corbin, 1990) and the 4-step methodology of Wester and Peters (2004). This methodology indicates to first explore the data to get an overview of all farmers' answers to the questions asked. Then, identification of the main themes or concepts and categorizing the farmers within these concepts. Finally, the themes were compared with each other and were integrated to formulate a theory based upon the transcribed interviews. To facilitate the analysis, a coding system was developed in which the interviews received a unique country code (The Netherlands, NL or Germany, DE), followed by the interview number, (1, 2, 3, etc.), and a letter (A, B, C) for the related theme or concept. The results are illustrated with quotes derived from the interviews. The quotes are translated to English as literally as possible.

RESULTS AND DISCUSSION

The aim of this study was to gain insight into factors influencing decision making on the duration of antibiotic treatment of clinical mastitis of dairy cows in The Netherlands and Germany. Seventy-two farms were contacted to participate in the study. The acceptance rate of participation was relatively high with 52% (38 farms were interviewed). Farm and mastitis treatment characteristics per farm within countries are shown in Table 2 and 3.

Reasons for extended treatment

On 37 of the 38 farms, clinical mastitis treatment was reported to be occasionally or frequently extended (Tables 2 and 3). Most farmers extend clinical mastitis treatment, either as a routine, for the majority of cases (n = 30) or only occasionally (n = 7). Only one Dutch farmer told the interviewer to never extend treatment. There seemed to be no relation between extending treatment and farm size, age, milk production level, BMSCC or milking system (Tables 2 and 3). The apparently generally applied habit of farmers to extend treatment of clinical mastitis suggests that this behavior is socially accepted, and that it is independent of other farm related variables. The main reasons for extended treatment retrieved from the interviews were that clinical symptoms were still present, fear for recurrence of clinical mastitis, other farmers' behavior, and veterinary recommendations.

Disappearance or recurrence of clinical symptoms.

The most frequently reported reason for extending antibiotic treatment was that clinical symptoms had not disappeared at the completion of the label protocol. This was most frequently reported in interviews in both countries (Germany 15/21, The Netherlands 12/17).

> *DE2:"At the moment I prepare a cow for milking I decide whether to stop treatment. If I still see flakes or watery milk, or the udder is still hard, we continue treatment. We usually need 5 to 7 days, if not cured then, we still continue treatment".*

From 4 farmers, 2 in Germany and 2 in The Netherlands, reported to extend antibiotic treatment as a standard therapy, irrespective of clinical symptoms. Fear that mastitis would recur as the main reason for extending treatment was reported more frequently in The Netherlands (n = 8) than in Germany (n = 1). At first glance, this seems to be a different reason for extending treatment than 'disappearance of clinical symptoms'. Both arguments are, however, related to bacteriological cure. Dairy farmers define cure as disappearance and non-recurrence of clinical symptoms. In many interviews, farmers expressed using their many years of experience in judging clinical signs as a basis for deciding on the best treatment. For them, relying on experience is more important than scientific evidence (Friedman et al., 2007). This empirical experience has been reported to be based on the ability to predict prognosis, based on former success and failure (Vaarst et al., 2002).

Previous research has shown farmers experience insecurity about how to treat mastitis (Jansen and Lam, 2012). This insecurity may fuel extending treatment as long as clinical symptoms are visible or when they recur. We hypothesize that giving the perceived best possible treatment makes farmers feel more secure about their treatment decisions and gives them the feeling of being a good farmer. This is perceived as a reward for extending treatment. Interestingly, none of the farmers expressed a concern on the increased costs of extended treatment due to more waste milk and higher antibiotic use, nor did anyone associate the potential hazard of prolonged exposure of pathogens to antibiotics, with potentially evoking antibiotic resistance. This indicates this disadvantage is not perceived relevant. If our hypothesis that extended treatment is rewarded through reducing insecurity on the treatment outcome is correct, this emotional reward apparently outweighs the associated higher costs and the potential risk of antibiotic resistance development.

Other farmers' behavior

Extending treatment of clinical mastitis was considered a 'thorough' approach by many interviewees. This shows that from an interviewees' point of view, animals are properly taken care of, as well as good stockmanship. The fact that 30 out of 38 interviewed farmers said so, indicates that this is a social norm that may also apply to other dairy farmers. This confirms the findings of a study on mastitis treatment decisions by Vaarst et al. (2002), showing that farmers are intrinsically motivated to control animal health and associate it with good stockmanship, which strengthens their pride. Additionally, Dutch dairy farmers were found to experience insecurity about the cure of a mastitis case (Jansen and Lam, 2012) which may even lead to perceived stress when treating clinical mastitis, as was explicitly expressed by one of the German interviewees.

> *DE9: "Nothing is more annoying than a cow with mastitis. The milk is gone and you have the stress of treatment."*

Another source of insecurity is the risk of recurrence of clinical mastitis after treatment, which may be encouraging extended treatment. The insecurity may make farmers sensitive for what other farmers do or do not do.

> *NL15:"You should treat it well and long. You should not stop the treatment too early. Some farmers always treat mastitis only for three days. But then mastitis will return. Therefore we concluded that you should extend the treatment, certainly if you deal with aureus, Staphylococcus aureus."*

The interviewed farmers in Germany and The Netherlands had clear perceptions of what a good farmer is and they clearly strive to be recognized as such by other farmers. This need for recognition is not a specific farmers' need, it is universal need for all humans (Taylor, 1992). Extended treatment was associated with not feeling guilty if mastitis recurs after all. It provided a sense of responsible behavior, as the following comment by of one of the German interviewees illustrates:

> *DE5: "We have a low BMSCC, a very good udder health and I want to keep it that way. I told them (other farmers, the vet) that when a cow gets clinical mastitis she must cure for 100% as soon as possible, because then mastitis does not recur and the cow is not able to spread the germs around. Only then I have peace of mind. Makes me feel good."*

Veterinary recommendations

Social influence occurs when people are looking for trustworthy information on specific subjects (Deutsch and Gerard, 1955). The interviewees perceived veterinarians and other advisors as reliable sources of technical information on mastitis treatment. In the interviews, farmers expressed that in the field of mastitis, they consider the information of the veterinarian as more valuable than that of other advisors. This confirms findings of other authors (Raymond et al., 2006, Jansen et al., 2008) that mastitis related information of local veterinarians is generally considered trustworthy and valuable.

> *NL8:"If you are on a dairy fair and talk to salesmen, you usually get a biased story about medication. They say their product is really great. Then you ask your veterinarian, what kind of medication is this? He knows more about the background of the product."*

This quote suggests that the social influence of the veterinarian is informational rather than normative. However, extending treatment is sometimes reinforced by veterinarians, which can be perceived as normative social influence. In 5 of the Dutch and 4 of the German interviews, the farmer spontaneously indicated that extending mastitis treatment was initiated because the herd veterinarian advised this.

NL6:"Ten years ago, a veterinarian from the drug company told me 3 tubes was sufficient, even when flocks were still visible. I have been in doubt on this, not sure whether this was good, but eventually it turned out to be OK. Last year my own vet advised me to use 6 tubes, he told me you always have to finish the course of treatment. He said, just use a double dose, that is better than an additional injection in the neck. This is the advice of the vet, these things change over time".

In both The Netherlands and Germany, pharmaceutical companies or distributors sell antibiotics for mastitis treatment directly to local veterinarians. Veterinarians generally make money on the products sold to farmers and thus, extending treatment of mastitis increases the income of veterinarians. We expected this to be mentioned in the interviews. Remarkably, dairy farmers never mentioned that veterinarians may also have a financial interest in advising to extend treatment of clinical mastitis. Because the veterinarian is perceived as a positive reference, their advice to extend treatment confirms the farmers' own idea that it is better to treat longer. The veterinarians' approval strengthens the farmers' perception that extended treatment is good stockmanship.

Attitude of treatment versus culling.

Some interviewees indicated that they extended treatment of clinical mastitis to prevent recurrence of clinical mastitis. We found interviewees used 2 different approaches of cows with recurrent mastitis. Eight of the interviewees considered it good stockmanship to be aiming for cows with greater longevity. For these farmers, to achieve that, treatment of clinical mastitis, including recurrent cases, needed to be as good as possible.

NL12: "For me, it is a priority that cows grow old. Without antibiotics, that is not possible. I think that farmers who are really careful with their cows, have older animals. And I also think that, on average, they use more antibiotics."

The other approach, expressed by 4 farmers, was to cull recurrent cases at an early stage;

NL7:"If you want to work economically, you should get rid of problem cows. Now, I cull cows if they are suffering from mastitis twice in one lactation. (…) In this way, after a number of years, a group is selected that can handle it. A group that does not suffer from mastitis."

This different attitude to treatment of recurrent mastitis cases may be the result of the diversity in the farmers' perception towards cattle (Bock et al., 2007). Bock et al. (2007), distinguished 'attached' from 'detached' farmers. Those who strive for greater longevity may be more emotionally 'attached' to their animals and may have a more cow oriented than those who have a more business oriented approach, cull cows at an earlier stage, and were described as more 'detached'. Generally speaking, dairy farmers' attitude may be an important predictor for antibiotic

use, as was earlier described for mastitis incidence (Jansen et al., 2009). Cow oriented farmers probably use more antibiotics and tend to more often extend treatment than business oriented farmers.

Clinical versus bacteriological cure

As described above, for farmers, the time needed for clinical cure seems to determine treatment duration. Guidelines for efficacy studies for registration of intramammary tubes, however, indicate bacteriological cure is considered the key parameter in evaluating success of treatment (CVMP, 2013). Clinical cure may occur at a later stage than bacteriological cure because the removal of the inflammatory debris from the udder may take more time than the killing of bacteria, and may be dependent on the pathogen involved (Schukken et al., 2011). This discrepancy between label claims and farmers perceptions of cure seems to contribute to a tendency to extend antibiotic treatment. Also, scientifically, it is unclear what the best parameters are to decide when to stop or when to extend a clinical mastitis treatment. For mild Gram-negative clinical mastitis cases, it has been shown that clinical improvement is a better indicator for bacteriological cure than clinical cure as such (Schukken et al., 2011). Thus, maybe, treatment should be stopped as soon as clinical symptoms diminish. More research on the evolution of clinical criteria, or on the development of practical cow-side diagnostic tests, indicating bacteriological cure during treatment is needed.

Social Reference Groups

People are continuously socially influenced by other people, by what they do, what they say and by what they do not say. What others do provides social proof regarding what is appropriate or inappropriate behavior in a given situation (Cialdini, 1984, Cialdini and Goldstein, 2004). Social influence occurs when people look for social norms of others (Turner, 1991). It is based on people's tendency to conform to positive rather than to negative expectations of others (Deutsch and Gerard, 1955). To reduce social uncertainty, people compare themselves to social norms of other groups (Abrams et al., 1990) and group pressure makes such beliefs stronger (Festinger, 1950). The closer to oneself a reference group is perceived to be, the higher the social influence of that group is on our own behavior (Festinger, 1954). By personal identification with a reference group, social norms are exchanged and serve as a reference for behavior.

In the interviews, 5 main stakeholders were identified as social groups that have normative influence on dairy farmers' clinical mastitis treatment decisions; their veterinarian, other advisors such as the nutritionist, other dairy farmers, meat producing farmers and society. Media, government, regulators and policymakers are all considered as part of society. Table 5 presents perceived positive and negative reference groups of the dairy farmers in the study. A positive reference group is a group that one accepts, identifies with, and feels psychologically attracted to (Turner, 1991). Table 5 shows that the veterinarian, other advisors, other dairy farmers and meat producing farmers are perceived as positive reference groups. These positive reference groups coincide with the groups that regularly come on farm. Farmers seem to identify with people close

to themselves, with whom they have face to face contact, resulting in positive reference and finally, social influence.

Table 5. Positive and negative reference groups related to antibiotic use for treatment of clinical mastitis by dairy farmers.

Negative reference groups	Positive reference groups
Meat producing farmers[1]	Meat producing farmers[2]
Other dairy farmers[3]	Other dairy farmers[4]
Society	Local veterinarian
	Other advisors

[1] In the context of quantities of antibiotics used, and of preventive versus curative use of antibiotics
[2] In the context of perception of society that farmers use too much antibiotics.
[3] Other dairy farmers that say they never have mastitis problems, and farmers that have a different approach in decision making of treatment versus culling.
[4] All other dairy farmers except those mentioned in 3.

A negative reference group is a group of people that one rejects and does not want to belong to (Turner, 1991). Table 5 shows that society was always perceived as a negative reference group by the interviewees. This indicates that the farmers in the study rejected the social norms of society, i.e. the societal perception that farmers use too much antibiotics.

We found that reference groups were not necessarily homogeneous and were not always judged in the same way by the interviewees. When other dairy farmers, who were generally perceived as a positive reference group, stated they never have mastitis problems in their herds, they were seen as untrustworthy and, consequently, as a negative reference group. In the same way, dairy farmers who have a different attitude towards treatment versus culling of recurrent mastitis cases, as described above, may also be perceived as a negative reference group. In the interviews, meat producing farmers, particularly in the pig, poultry and veal calf industry, were generally seen as a positive reference group because they were perceived as colleagues. However, they were perceived as negative with reference to antibiotic reduction programs. They were perceived to use much more antibiotics than dairy farmers and to use it for prevention of disease rather than for cure.

DE1: "Livestock farmers use too much antibiotics is what the press reports. Probably not in the dairy sector, but in the poultry sector. The poultry sector cannot survive without antibiotics. If they see a sick animal, they have to treat all animals with antibiotics to improve growth. We, the dairy farmers, only use antibiotics to treat sick individual animals."

Approximately 75% of the interviewees indicated that, according to them, the meat producing farmers are the cause of antibiotic resistance, not the dairy farmers.

DE3: "It is how it is presented. The people are often told how bad things are in feedlots, in poultry farming, or in pig farming, the extreme-large scale animal husbandry. This is always bad for us, we are linked to this image, even though it is not so bad in dairy farming. In dairy farming, the milk is checked before consumption and the consumer does not have to fear antibiotic residues. This is different for meat. These large scale farms cannot survive without

antibiotics. And I think sometimes, the dairy sector has to ignore this a bit, when using mastitis antibiotics. Well, when you see the total consumption of antibiotics in food animals, a lot of antibiotics are used. But we use far less than meat producing farmers."

People, including dairy farmers, generally categorize themselves by identifying or not identifying themselves with social norms of groups of people (Turner, 1991). By showing their approval or disapproval towards norms of other farmers, such as expressing to have mastitis problems, to readily treat recurrent cases of mastitis or to use antibiotics preventively, they categorize themselves and thereby identify who they are and what they think. Because other dairy farmers generally are a positive reference group, very similar to themselves, dairy farmers want to conform to the norms of other dairy farmers (Festinger, 1954). This means that dairy farmers are sensitive to what other dairy farmers say and do:

DE9: "I think there is not a lot of difference in the way farmers treat mastitis."

The interviews clearly reveal an important social norm: to try and be a 'good farmer'. A good farmer is someone who takes good care of his animals. Expressing problems, concerns and uncertainty may be (mis)interpreted by other dairy farmers as signs of being a 'bad farmer'.

NL7:"Sometimes I talk about mastitis problems with friends. But you do not tell everybody. You will never say to everybody; 'Hey guys, listen, I really have a lot of cows suffering from mastitis.' You know for yourself, if you just ask someone, they do not always tell the truth. If you know one another well, you may talk about it. Then you ask, how do you cope with it? You will not tell a complete stranger that you have a lot of problems with mastitis. Then your good workmanship is questioned."

According to the interviewees, admitting that your cows are suffering from mastitis may cause other farmers to think you are a 'bad farmer', which in turn increases the risk of social sanctions. To avoid this from happening, many of the interviewed farmers appear to be inclined to not discuss their mastitis problems with other farmers.

Social Influence of Society

Conforming to group norms is reinforced by the disapproval of negative reference groups. Such group norms where people express whether other people approve or disapprove, are called injunctive norms (Turner, 1991). All dairy farmers disapproved the injunctive norm of rigorous reduction of antibiotic usage in livestock farming being imposed by society in general. Outside the inner circle of positive referenced farmers and veterinarians, the external regulators (society), such as the government, are seen as a negative reference group with a different injunctive norm (Table 5).

DE9:"I will never change my treatment strategies just because my neighbor from down the road, who votes for the 'environmental friendly' political party, will come here every day, stick his long neck in my barn, and say: 'what is that you have in your syringe?'."

Not agreeing with society's norm results in a polarization, mainly because society and the associated animal protection organizations are perceived as not recognizing good stockmanship.

NL7: "They imagine that farmers only spend their time spreading manure with a slurry tank or applying antibiotics to treat animals. They don't know any better. I think society does not really understand that it is sometimes better for the animals to be treated with antibiotics."

The perceived norms of society were rejected by the respondents, because they were seen as unjustified. Moreover, it was perceived as disrespectful towards farmers' professionalism and intrinsic need to take good care of their animals. The interviewed farmers perceived that societies' negative perceptions of them using too much antibiotics fuels the antibiotic debate, while they believed they only use antibiotics to take good care of their animals.

NL10: "*They talk about sustainability. The cows should live as long as possible. And now, they are culled earlier. Because you cannot treat properly. They talk about sustainability."*

If one wants to influence the antibiotic usage of dairy farmers, it is important to realize how the sender is referred to by the receiver of the message. For societal actors and regulators, that likely is as a negative reference group, which probably has consequences for communication to be effective. To our opinion mutual open communication, showing understanding and respect in a dialogue, is warranted to be successful. Although there seem to be shared values, such as taking good care of animals, there is a large, seemingly unbridgeable emotional gap, between society and dairy farmers, with farmers feeling misunderstood and not recognized for their good stockmanship. To overcome that gap, one should not only try to improve mutual understanding through dialogue based on shared values, but farmers also need to work more in compliance with societies perceptions (De Greef et al., 2006).

Differences between Germany and The Netherlands

Social influences on farmers with respect to antibiotic treatment showed the same pattern in Germany and The Netherlands. Also, both governments recognize that the use of antibiotics in livestock has to be reduced and have banned its preventive use. There are, however, differences. At the time of the interviews in The Netherlands, meat producing farmers as well as dairy farmers actually have to reduce the use of antibiotics. The German authorities, however, have chosen to start their antibiotic reduction program in poultry, followed by pigs and finally in cattle. The interviews indicate that the antibiotic approach in The Netherlands is considered unfair by the Dutch dairy farmers, evoking negative feelings towards society.

NL10: "The contribution of the dairy sector is only a very small part of the use in the entire livestock sector. So, in fact, the dairy sector is dragged along in the policies of antibiotic reduction, which is undeserved".

Because the antibiotic reduction program in Germany started on other types of farms, German dairy farmers are not yet affected. Nevertheless, the German interviewees seem to experience some social pressure to critically look at their own antibiotic use.

DE1: "Yes, society influences my decisions on the use of antibiotics. It is better to already work a bit towards less antibiotic use than to be forced afterwards. Because then you might be unable to comply. But when they take antibiotics completely away from us, I would consider that as unjustified".

The knowledge of obligatory antibiotic reduction in the near future allows dairy farmers in Germany time to voluntarily change their behavior. Tighter norms, not yet being imposed on them at the time of the interviews, seemed to result in less negative emotional impact and in less negative feelings between dairy farmers, meat producing farmers and society. This more mild attitude towards antibiotic reduction in Germany, may change at the time German dairy farmers are directly affected and actually have to change their behavior regarding the use of antibiotics.

Reflections on the Study

In the study 38 farmers were interviewed in 2 different countries. This is only a small sample and probably does not reflect the entire population. This study is therefore exploratory and additional research is recommended to verify the findings. Because approximately half of the approached dairy farmers were willing to participate, selection bias may have occurred, potentially influencing the results. Because discussing antibiotic use is a sensitive topic, those farmers that did participate in the study, may have expressed a more positive or a more negative opinion on antibiotic reduction than average. Nevertheless there are some lessons that can be learned from this study on social influence of the different reference groups and established norms of mastitis treatment, based on trying to be a good farmer.

Perceptions on the use of antibiotics in clinical mastitis may be different in different parts of Europe. It would be interesting to repeat this study in other EU countries, to explore whether our findings are more universal and could help in an international approach in optimizing antibiotic use for clinical mastitis in dairy cows.

Although extending treatment of clinical mastitis in relation to efficacy and prudent antibiotic use is debatable, it is the social norm. At the national level, the development of antibiotic resistance in livestock was found to be positively related to the quantity of antibiotics used (Chantziaras et al., 2014). The consequences of extending treatment of individual mastitis cases, however, are not clear. Currently, farmers treat clinical mastitis cases longer than indicated on the label , because it is perceived to be the better for their animals. Prudent antibiotic use warrants more research, not only on the efficacy of antibiotic treatment, but also on the consequences of antibiotic treatment for the development or prevention of antibiotic resistance in bacterial pathogens.

CONCLUSIONS

The aim of this study was to gain insight into the social influence on decision making on the duration of antibiotic treatment of clinical mastitis of dairy cows. This study clearly shows that farmers try to be a ‘good farmer’ and as such, are very sensitive towards social norms of other farmers. A good farmer extends treatment because it is the social norm. Farmers express their insecurity regarding what it entails to be a good farmer in relation to mastitis treatment and whether their treatment is effective and prevents mastitis from recurring. This insecurity makes them more sensitive to influence of positive reference groups such as other farmers, their veterinarian and results in a social norm of extending treatment. Conforming to other farmers injunctive (‘what

ought to be') and descriptive ('what is done') norms and the veterinarians' informational norm that extended treatment is better, results in social approval, reinforcing farmers own perceptions that, in case of doubt on cure, treatment should be extended. The perceived reward for dairy farmers, especially more 'cow oriented' farmers, of extended treatment is the reduction of insecurity by social approval of positive reference groups. This perceived social reward clearly outweighs the penalty of higher costs of more waste milk and more antibiotic use. Changing behavior of extended treatment will likely result in social sanctions from positive reference groups for not complying to injunctive norms and will therefore be difficult to achieve. We hypothesize that legislation, if doable and controllable, will force farmers to reduce antibiotic usage. However, to change social norms of 'thorough' treatment, new evidence based information, communicated by a positive reference group, i.e., the local veterinarian, will be necessary. Thus, more research is needed on the efficacy of antibiotic treatment on cure and on recurrence of clinical mastitis. Practical cow-side criteria indicating when to stop treatment, such as a reduction in clinical signs or a reliable and easy to perform cow-side test, would help in making optimal decisions on continuation of treatment.

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CHAPTER 8

General Discussion

The aim of studies described in this thesis is to evaluate the bacteriological, clinical and economic efficacy of extended antibiotic treatment of persistent mastitis during lactation compared to standard treatment. An additional aim was to explore the social influence on farmers with respect to their choices on duration of antibiotic treatment of mastitis.

The bacteriological and clinical efficacy of extended mastitis treatment was studied in 3 large clinical field trials (Chapter 4, 5, 6). Additionally, economics of subclinical mastitis treatment was evaluated (Chapter 2 and 3) and finally, social influence on farmers in clinical mastitis treatment was studied (Chapter 7). In this final chapter, results from preceding chapters, combined with hitherto unpublished data, are discussed. The main focus of this chapter will be on practical implications of the choices related to extended therapy for the dairy farmer and the veterinary practitioner.

Incidence and indicated reasons of extended treatment of clinical mastitis

Feedback from the field suggests treatment of clinical mastitis cases is frequently repeated after initial treatment according to the label claim, thereby extending the duration of treatment. To confirm this feedback, clinical mastitis treatment of 203 dairy farms of 3 different veterinary practices in The Netherlands was evaluated. These 3 veterinary practices were located in the north (82 dairy herds), in the east (60 dairy herds) and in the south (61 dairy herds) of the country. It was found that in 66% of all recorded treated clinical mastitis cases evaluated, intramammary antibiotic treatment was extended beyond the duration indicated on the label. The percentage of cows with extended intramammary treatment ranged from 48% to 80% for the 3 veterinary practices. Ninety percent of farmers indicated to generally extend treatment because clinical symptoms have not fully disappeared. Other reasons that were indicated are; to prevent mastitis recurrence, as a habit, lack of awareness of labeled treatment duration, and based on advice received from the herd veterinarian.

If these 203 farms are somewhat representative, it seems that in The Netherlands, a large proportion of treatments is repeated after initial label treatment, thereby extending treatment duration. To judge whether this is a typical Dutch phenomenon, 7 experts in the field of mastitis, from different EU countries, were asked to estimate the duration of treatment of clinical mastitis in their own country. Except Denmark, most experts in these countries confirmed the Dutch findings that treatment is extended in the majority of cases (Table 1). In Denmark the average duration of treatment is similar to other EU countries, but label claim indications for treatment duration are longer than in other countries, resulting in treatments according to label claims. The most important reason for extended treatment indicated by 5 of the 7 experts is ‘symptoms have not disappeared’, only the UK and Denmark indicate another reason as most important to extend treatment.

Table 1. Estimated duration of clinical mastitis treatment by 8 EU experts in the field of mastitis; Denmark (DK), Italy (I), United Kingdom (UK), Belgium (B), Spain (S), Germany (De), The Netherlands (NL) and France (F).

	DK	I	UK	B	S	De	NL	F
Average treatment duration (days)	3-4	4-5	4-5	3-4	3	3-4	3	3-4
Treatment duration according to label claim (days)	3-5	1-2	1.5-3	1.5-2	1.5-3	1.5-2	1.5-2	1.5-2
Frequency of repeated treatment after initial treatment to label claim (%)	5	>50	>50	>50	>50	>50	66	50
Most important reason for extended treatment	a	b	c	b	b	b	b	b

a Advice by the veterinarian, in case of frequent mastitis recurrence
b Clinical symptoms have not disappeared
c To prevent mastitis recurrence

These data indicated that, throughout the EU, clinical mastitis treatment is frequently extended, despite a mounting pressure to reduce the use of antibiotics and the consequences such as increased costs of discarded waste milk, higher antibiotic use, and the potential risk for the development of antibiotic resistance. Farmers apparently have a rationale to extend treatment that outweighs the higher costs and potential risks. Such reasons may be higher efficacy of treatment or economic gains. Thus, important aspects for further consideration on the use of extended treatment are efficacy, economics and risk for antibiotic resistance development. In addition, social influences related to extended treatment will be discussed, because decision making is not always based on rational arguments, but on what people hear or observe from peers or other influencers (Cialdini, 1984).

Efficacy of treatment at cow level

Efficacy of treatment can be evaluated at different levels. The most obvious is efficacy at the quarter or cow level using bacteriological and clinical cure or the decrease in SCC. Efficacy of treatment can also be evaluated at the herd level, taking into account indirect effects of cure or non-cure with regard to transmission of pathogens to other cows in the herd. Herd level udder health management measures affect treatment efficacy too, because after initial cure, cows may get re-infected in poorly managed herds. In such herds, cure and immediate re-infection is often interpreted as non-cure.

The most important parameter when judging treatment efficacy traditionally is bacteriological cure. It is a parameter that can be more or less objectively measured in the laboratory. Bacteriological cure is dependent on the host (DeLuyker et al., 2005), the pathogen (Bradley et al., 2009, Swinkels et al., 2014) and the antibiotic (Shpigel et al., 1997, Bradley et al., 2009), including application route (Hillerton and Kliem, 2002, Barkema et al., 2006) and duration of treatment (Truchetti et al., 2014).

Six peer reviewed papers on extended treatment of clinical mastitis and 5 peer reviewed papers on extended treatment of subclinical mastitis, showed an improved bacteriological efficacy of a longer duration of treatment for up to 5 or 8 days, versus a standard treatment

duration of 1 to 3 days (Table 2). Most of these studies focus on a single mastitis causing pathogen, usually *Staphylococcus aureus* or *Streptococcus uberis*, because these pathogens are generally considered to cause persistent infections and therefore are difficult to cure. Five studies evaluated treatment of mastitis cases irrespective of the underlying pathogen. Three of these studies showed an improved overall bacteriological efficacy of extended treatment across pathogens (Krömker et al., 2010, Truchetti et al., 2014, Steele and McDougall, 2014) while 2 did not (McDougall et al., 2014, Swinkels et al., 2014).

Table 2. Literature overview of studies reporting bacteriological efficacy of extended (5-8 days) versus standard duration (1-3 days) of antibiotic treatment of subclinical, clinical and persistent clinical and subclinical mastitis.

	Staph. aureus	Strep. uberis/ dysg.	Across pathogens	Antibiotic(s)
Clinical mastitis				
Jarp et al., 1989	++[1]			Procain penicillin / dyhydrostreptomycin
Pyörälä and Pyörälä, 1998	+			Procain penicillin, spiramycin, (enrofloxacin)
Sol et al., 2000	++[1]			Miscellaneous
Oliver et al., 2004a		++[2]		Ceftiofur
Krömker et al., 2010		++	++	Lincomycin/neomycin
Truchetti et al., 2014	++		++	Ceftiofur
McDougall et al., 2014			00	Amoxycillin/clavulanic acid
Subclinical mastitis				
Gillespie et al., 2002	++	+		Pirlimycin
Oliver et al., 2003		++[2]		Pirlimycin
Oliver et al., 2004b		++		Ceftiofur
Roy et al., 2009	++			Cephapirin
Steele and McDougall, 2014			++	Penethamate hydriodide
Persistent clinical mastitis				
Milne et al., 2005		++		Procain penicillin and dyhydrostreptomycin or cefquinome
Swinkels et al., 2013 (Chapter 4)	00			Cefquinome
Swinkels et al., 2014 (Chapter 6)		++	00	Cefquinome
Persistent subclinical mastitis				
DeLuyker et al., 2005	++			Pirlimycin

[1] only for β-lactam negative strains, [2] Experimental trial, ++ extended treatment significantly higher bacteriological cure ($p<0.05$), + a trend ($p<0.2$), 00 extended treatment not different from control ($p\geq0.2$).

Three studies were published on the effect of extended therapy of persistent clinical mastitis. It was found to be only beneficial in cases caused by streptococci (Milne et al., 2005; Swinkels et al., 2014), but not across pathogens (Swinkels et al., 2014) nor in cases caused by *Staph. aureus* (Swinkels et al., 2013). It has also been described that extended treatment is only beneficial for certain strains within a bacterial species, i.e. for β-lactamase negative *Staph. aureus* (Jarp et al., 1989, Sol et al., 2000). We have not been able to confirm that finding in Chapter 4.

In the field, specifically in clinical mastitis cases, the underlying pathogen is usually unknown. Benefits of extended treatment, irrespective of the underlying pathogen, as shown by Krömker et al. (2010) in an univariate analysis, could not be confirmed in Chapter 6. We have to realize, however, that the logistic regression model in Chapter 6 was built to show differences between and not across pathogens. The absence of a beneficial effect on bacteriological cure across pathogens after extended treatment, as found in Chapter 6, has recently also been observed in a comparable trial by McDougall et al. (2014). In that study, as well as in ours, a difference between bacteriological and clinical cure was described. Possibly, bacteriological culturing is not the optimal predictor for eventual recurrence of clinical mastitis.

The clinical field studies in Chapter 4 and 6 of this thesis are among the first published peer reviewed studies that do not confirm that extended treatment improves bacteriological cure, and indicates that extended treatment protocols may not always be beneficial. The studies in Chapter 4 and 6 differ from other studies in the sense that the selection of clinical cases was focused on persistent infections. The study in Chapter 4 is mainly conducted on *Staph. aureus* problem farms, likely to contain a high amount of persistent intramammary infections (**IMI**). In Chapter 6, only persistently infected cows with 2 consecutive monthly elevated cow SCC above 200.000 cells/ml before clinical mastitis occurrence were selected for treatment. The lack of bacteriological efficacy, as found in Chapter 4, and across pathogens in Chapter 6, suggests that there may be a difference between clinical mastitis and persistent clinical mastitis with respect to the added value of extending antibiotic treatment.

Not all antibiotics are evaluated for all mastitis pathogens and for all different types of IMI. Different situations are not comparable and therefore extended treatment of clinical mastitis should not be advocated as a routine treatment protocol in IMI in all situations. Extended treatment of persistent clinical mastitis should be evidence based and limited to specific antibiotics, specific management situations, and to specific indications, such as *Strep. uberis* IMI, as was shown in Chapter 6. Based on that, farms using on-farm culture programs may benefit from these specific indications for extended treatment (Lago et al., 2011a, b).

Publication bias

An overview of studies on extended versus standard treatment of mastitis is presented in Table 2. This overview clearly shows that, if the studies described in Chapter 4 and 6 and the study of McDougall et al. (2014) are not taken into account, literature shows that extended treatment has better bacteriological results than standard duration of antibiotic treatment. We have to realize, however, that publication bias is a threat to the validity of such conclusions. The tendency of researchers, editors, and pharmaceutical companies to select positive results for scientific reports, may result in a misleading bias in the overall published literature, leading to incorrect, usually over-optimistic, conclusions (Thornton and Lee, 2000). Such bias could come from many sources, one of them being that the results might not have been interesting enough for researchers or reviewers, or that studies are sponsored by pharmaceutical companies that don't see a commercial benefit in publishing reports without positive results (Thornton and Lee, 2000). Halasa et al. (2009) showed in a meta-analysis of dry cow treatment efficacy studies

that according to the fill and trim method as discussed by Duval and Tweedie (2000), 7 studies, showing no efficacy of dry cow treatment were likely missing, indicating a bias in the assumed effect of dry cow treatment to prevent *Staph. aureus* infections. Although these statistical methods are based on assumptions, they do indicate the potential publication bias. Likewise, publication bias may have influenced the peer reviewed literature on extended versus standard treatment of clinical mastitis. The studies reported in Chapter 4 and 6 may be among the 'missing studies' reporting that extended treatment is not always better than standard treatment. The fact that the studies in Chapter 4 and 6 are among the first studies that do not see an overall benefit of extended treatment, may have many causes. One of them is publication bias.

Role of the antibiotic

In Chapter 4, 5 and 6 cefquinome was used for treatment of clinical mastitis. To reduce the possible contribution to resistance in human pathogens, the use of cephalosporins such as cefquinome is under a mounting pressure in the EU (CVMP report, 2009). Although there is discussion on the use of cephalosporins in mastitis therapy, the extended treatment findings described in Chapter 4, 5 and 6, are of value. These products are extensively used in many parts of the world and therefore need evidence based recommendations. Additionally, the findings can possibly be extrapolated to other time dependent broad-spectrum β-lactam antibiotics, a class of antibiotics that also is widely used in the Netherlands for mastitis treatment. Sol et al. (2000) found that the type of antibiotic used for treatment of clinical *Staph. aureus* mastitis did not have a major effect on bacteriological cure. Others, however, showed the antibiotic used did make a difference for bacteriological efficacy (Bradley et al., 2009). Cephalosporins, like other β-lactam antibiotics, macrolides and lincosamides (pirlimycin), belong to the group of time dependent antibiotics that need a certain amount of time above MIC to effectively kill bacteria (Prescott et al., 2000, Toutain et al., 2002). They contrast with concentration dependent antibiotics, like aminoglycosides and fluoroquinolones, that need a high enough peak concentration above MIC to be effective (Prescott et al., 2000, Toutain et al., 2002). Almost all studies presented in Table 2 evaluated time dependent antibiotics. It is likely that extended treatment is only effective using time dependent antibiotics, such as β-lactams, and not for concentration dependent antibiotics such as aminoglycocides and fluoroquinolones. This probably explains why studies using only concentration dependent antibiotics are lacking in the overview in Table 2.

Clinical cure

Because mastitis is painful and affects animal welfare, clinical cure is an important aspect of treatment. In addition, farmers aim for fast clinical cure to bring the cow back into production as soon as possible. Although researchers traditionally consider bacteriological cure as the most important goal of treatment, clinical cure is what farmers see and judge. Not surprisingly, farmers use their empirical experience in judging clinical signs as a basis for treatment or re-treatment decisions (Vaarst et al., 2002).

As indicated in Table 1 of this Chapter and the interviews in Chapter 7, the duration of treatment seems to be mainly guided by the time it takes for clinical symptoms to disappear. This shows that in the field, persisting or recurring clinical signs, provoke extended treatment, in the expectation of a more effective elimination of clinical signs. The study in Chapter 5 confirms that, based on farmers' observations, extended treatment is better because it significantly reduces persistence and recurrence of clinical signs, as was also described by McDougall et al. (2014).

Guiding treatment duration by the disappearance of clinical symptoms would be justified if this was a reliable indicator for the disappearance of mastitis causing bacteria. A correlation between clinical and bacteriological cure is theoretically expected because after the disappearance of bacteria, inflammatory signs are likely to subside and resolve. The decision to prolong treatment is often taken in the early stages of treatment. In Chapter 6 was described that after 1.5 and 5 days of treatment around 15% and 60% of cases, respectively, were clinically cured. The eventual bacteriological cure, at day 14 and 21 after the start of the treatment, was around 72% and 79% of cases, respectively, and did not differ between treatment groups. These findings show clinical cure at day 1.5 is considerably lower than eventual bacteriological cure and that the presence of clinical symptoms at day 1.5 is not a good indicator for prolongation of treatment. Therefore, the decision to continue treatment beyond 1.5 days based on the presence of clinical symptoms at that time-point, does not seem the optimal approach. Others have suggested that clinical improvement rather than clinical cure as such is a good indicator for eventual bacteriological cure (Schukken et al., 2011). However, in this study, clinical improvement was measured at day 14 after treatment, long after the moment farmers decide to extend treatment or not. In our studies in Chapter 4 and 6 only the absence or presence of clinical signs (clinical cure) was measured at day 14 or 21 after treatment and not clinical improvement. Therefore the approach of Schukken et al. (2011) could not be evaluated in our data. There is an obvious need for cow-side tests during the first days of treatment, that reliably indicate when to stop treatment. In future research, it would be valuable to study the correlation between the quantitative evolution of bacteria in milk of affected quarters in the first days after treatment and the associated clinical symptoms.

In this thesis, cow related factors have been described to be indicative for clinical cure. Firm udders at palpation at the time of diagnosis were associated with a lower probability of clinical cure, in case of *Staph. aureus* mastitis (Chapter 4). Also, lactation stage, specifically the first 100 DIM, was found to be associated with a lower probability of clinical cure (Chapter 6). Cows in the first 100 days after calving may be in a negative energy balance, affecting host immunity (Kehrli et al., 1994). A lower immune status may slow down the ability of the udder to clear an inflammation. This was also described by the respondents in the interviews in Chapter 7, who expressed that the optimal treatment duration of clinical mastitis is not standard, but is different for different cows.

The study of Schukken et al. (2011) also suggests that clinical improvement is pathogen dependent, showing a significantly lower clinical improvement in mastitis caused by *E. coli* and *Klebsiella* spp. compared with cows infected with *Enterobacter cloacae.* In chapter 6, however, no significant pathogen related factors were found to influence clinical cure after treatment. Although the evolution of clinical signs may or may not be pathogen dependent, there seems to

be a pathogen dependent correlation between clinical and bacteriological cure at 14 and 21 days after treatment. In this thesis, a clear discrepancy between bacteriological and clinical cure was found for *Staph. aureus* IMI in Chapter 4, showing overall clinical cure (±70%) to be considerably higher than the overall bacteriological cure (±30%). For streptococci, overall clinical cure (±99%) was also higher, but more in line with bacteriological cure (±75%) than for *Staph aureus*. These findings suggest that, specifically for *Staph. aureus*, clinical cure at day 14 after treatment is much higher than bacteriological cure and thus, is not a good parameter of bacteriological cure. More research is needed to determine cow and pathogen related risk factors for clinical signs to resolve.

Efficacy at the herd level

Effective treatment of persistent mastitis has indirect herd level effects as described in Chapter 2 and 3 and in the work of Barlow et al. (2009, 2013). Bacteriological cure after antibiotic treatment of persistent mastitis prevents the spreading of mastitis causing bacteria to herd mates. This preventive effect of antibiotic treatment is a major determinant of the costs of clinical (Down et al., 2013) and subclinical mastitis treatment as described in Chapter 2 and 3. However, if successful treatment of mastitis is not accompanied by good udder health management, initially cured IMI will likely re-infect. Re-infection of initially cured quarters not only occurs because of increased infection pressure in the herd, but also because some quarters are more sensitive to re-infection (Zadoks et al, 2001). This may be the reason that no bacteriological effect of extended treatment of *Staph. aureus* was found in the study described in Chapter 4. Re-infection with *Staph. aureus* may have resulted in perceived low bacteriological cure rates and absence of higher efficacy of extended treatment. Using strain typing methods would have allowed us in a proportion of cases to distinguish between non-cure and re-infection (Barlow et al. 2013). Such molecular epidemiological studies would result in a more precise estimate of bacteriological cure. Re-infection is a likely scenario because as described in Chapter 4, re-infection with other pathogens than *Staph. aureus* after cure was also high (42%), confirming that, as stated above, extended treatment protocols have to be accompanied with good udder health management measures that prevent cow to cow transmission, in order to be effective (Barlow et al., 2009). Thus, in the control of *Staph. aureus* mastitis, farmers and their herd veterinarians should focus on the prevention of new IMI, by preventing cow to cow transmission (Fox and Gay, 1993) and increasing host resistance, before they consider to routinely extend treatment of clinical mastitis.

Economic efficacy

Economic pressure on dairy farms is and will remain large. Only farms that work economically are able to survive. Therefore, key parameters with regard to efficacy of antibiotic treatment of persistent mastitis during lactation is not only bacteriological and clinical cure but also economic profitability. Including indirect effects of treatment of subclinical IMI of *Staph. aureus, Strep. uberis* and *Streptococcus dysgalactiae*, such as reduction of pathogen transmission after cure, shows that treatment of subclinical mastitis is economically beneficial in many cases. The economic

benefit of extended therapy is only present for *S. aureus* IMI in herds with a high cow to cow transmission rate as indicated by a high R-value (Table 3), representing a contagious outbreak situation. As described in Chapter 2 and 3, the economic efficacy of subclinical mastitis therapy is therefore embedded in the epidemiology of mastitis pathogens in the herd and is therefore herd dependent.

If management measures, including treatment, become successful, and a herd transfers to a low transmission rate situation, extended treatment often is no longer economically beneficial, especially for herds where *Staph. aureus* is the predominant pathogen (Table 3). This shows that also from an economical perspective, extended treatment should always be combined with management measures directed at lowering transmission of infections, i.e. through decreasing infection pressure. Herd veterinarians should therefore not only focus on advising farmers on treatment and culling decisions of already infected animals but also emphasize the need for preventive management by optimizing feeding, housing and milking practices. If this kind of preventive measures is not taken, the indirect preventive effects of treatment decreases, leading to a new infection rate beyond an optimum transmission parameter (Barlow et al., 2009) and thus an unfavorable result of extended treatment. The 'treatment and culling only' approach then not only leads to limited results and high costs, but also to unnecessary use of antibiotics. Such practices are to be avoided in EU societies that call for sustainability and prudent use of antibiotics.

Table 3. Economic benefit (€) of 3 versus 8 day antibiotic treatment of persistent subclinical mastitis in high and low transmission rate herds for *Strep. uberis or Strep. dysgalactiae* (Chapter 2) and *Staph. aureus* (Chapter 3).

Pathogen	3-day treatment	8-day treatment
Strep. uberis or Strep. dysgalaciae		
Low transmission rate (R[1]=0.21)	+11.62	- 21.83
High transmission rate (R=1.4)	+68.60	+58.62
Staph. aureus		
Low transmission rate (R=0.32)	-21.12	-57.70
High transmission rate (R=5.3)	+95.62	+142.42

[1] R= reproduction ratio

Prudent use of antibiotics

Prudent antibiotic use requires evidence based justification of treatment protocols. The current situation in the EU is that the treatment of the majority of clinical mastitis cases is extended based on the perception that a longer duration of treatment is always better. This thesis, specifically Chapter 4 and 6, provides evidence to challenge this perception and provided evidence that extended treatment may not always be necessary and is only indicated for specific cows, pathogens or farms.

A correlation between quantities of antibiotic use at the country level, and antibiotic resistance development has been described in food producing animals (Chantziaras et al., 2013) as well as in human medicine (Bronzwaer et al., 2002). These data suggest that from a prudent antibiotic

use point of view, we want to use as few antibiotics as possible. It is, however, not so obvious that using less antibiotics in individual treatments, such as mastitis treatments, also results in less antibiotic resistance. Scientific evidence to support a widespread, emerging resistance among mastitis pathogens to antibacterial drugs is currently lacking (Erskine et al., 2006).

Cefquinome was applied intramammary in Chapter 4, 5 and 6. An increased resistance to fourth generation cephalosporins would be of potential concern for both veterinary and human applications (Locatelli et al., 2009, Pfeifer et al., 2010). However, it is not clear whether development of antibiotic resistance occurs if application of antibiotics is restricted to the mammary microbiome. Systemic absorption of cefquinome, after intramammary treatment, was shown to be negligible in healthy and subclinically infected cows (Zonca et al., 2011).This may, however, be different for other antibiotics, for parenteral treatment and also, in severe mastitis cases, when the blood-milk barrier is disturbed.

In human medicine in The Netherlands, many physicians tell people to 'complete the full course of treatment' in order to improve cure and reduce the risk of antibiotic resistance. In the last decade, however, several papers in human medicine show that shorter courses of treatment have comparable treatment efficacies as longer duration of treatment, i.e. in cases of bronchitis (El Moussaoui et al., 2008), urinary tract infections (Fitzgerald et al., 2012) and pneumonia (Hedrick et al., 2007). The relation between duration of treatment and bacteriological cure appears to be dependent on the nature of the underlying infections and host immunity (Geli et al., 2012). These authors suggest that for most infections, the optimal antibiotic dosing strategy for clinical treatment may not always be the optimal strategy for preventing the spread of resistance. They conclude that for the duration of antibiotic treatment there is no 'one size fits all' solution, although generally, shorter durations of treatment are to be preferred over longer durations. Transferring this approach to the treatment of persistent bovine mastitis during lactation means that, unless there is evidence of the added value of extended treatment, standard duration of treatment is recommended.Our data and the literature suggest that from a bacteriological perspective, extended treatment of time dependent antibiotics would be indicated for clinical mastitis caused by streptococci. The bacteriological success of extended treatment of clinical mastitis caused by persistent *Staph. aureus* may be successful in some cases, but is highly dependent on the bacterial strain, the cow and the farm. These suggested indications for extended treatment should be further confirmed and should be registered on the label of the antibiotic products. Rethinking the use of antibiotics in treating bacterial infections in animals is essential for farmers and veterinarians to maintain a society provided 'license to produce'. Further studies on rational use of antibiotics in veterinary medicine are warranted, focusing on minimal use of antibiotics at the national level, while leaving room for optimal use at the individual level. In the case of the dairy industry, this should focus on the treatment of specific pathogens in specific situations, including attention for cow and herd factors.

Social influences

Udder health management on dairy farms is better explained by attitudes of farmers than by their specific knowledge of technical veterinary aspects (Barkema et al., 1999, Jansen et al.,

2009). In the interviews described in Chapter 7, farmers mostly told the researchers they extend treatment because symptoms have not disappeared or because they fear mastitis will return. Irrational arguments, such as the desire to be seen by others as a good farmer and subsequent compliance to the social norm, are very important for the implementation of treatment protocols. Recommended treatment protocols by positive social reference groups such as other farmers and the veterinarian result in social approval. Social approval reduces insecurity of being perceived as a poor farmer and thus, extended treatment is emotionally rewarded. This subjective social reward apparently outweighs other, more objective arguments against extended treatment such as higher costs, more waste milk and more antibiotic use. In this thesis extended treatment of persistent mastitis was shown to be not always economic and not always effective. Despite these and other (Steeneveld et al., 2011) rational economic reports to not routinely extended treatment of mastitis, farmers have collectively adopted this practice. Apparently, it is not only rational economic arguments that are decisive for decision making in mastitis treatment. Farmers (and others) appear to do what feels right and what is socially acceptable. However, where this behavior is not always in compliance with prudent antibiotic use guidelines, it may fuel perceptions from society that farmers use too much antibiotics. Nevertheless, the thought that extended treatment may not always be necessary, will likely evoke negative responses by farmers and others, because it is against the social norm of 'being a good farmer' who treats mastitis thoroughly. It is likely that in order to change farmers' perceptions of treatment protocols, local veterinarians, and opinion leading farmers, perceived as good farmers, should play a key role in challenging the social norm of a good farmer.

CONCLUSIONS

This thesis shows that extending antibiotic treatment of persistent bovine mastitis during lactation is widely applied but its efficacy may be limited to specific antibiotics, cows, pathogens and farms. The choice to extend treatment is not only based on rational arguments but also on subjective habits and beliefs. Bacteriological, clinical and economic efficacy of extended treatment of persistent clinical and subclinical mastitis is not always better than standard treatment. Efficacy of antibiotic treatment is influenced by the quality of preventive udder health management measures. Thus, veterinarians and farmers should focus on udder health management aspects rather than on (extended) treatment protocols alone. In the field there is a need for a tool to decide on the optimal duration of treatment of mastitis from the perspective of bacteriological, clinical and economic efficacy. Quantitative data on the relationship between duration of therapy and the possible consequences for antibiotic resistance development are essential as currently arguments in either direction can be made. Therefore, future research should focus on developing reliable, cheap and easy to perform, preferably cow-side, tests to determine the optimal moment when to stop treatment and on quantitative studies that relate duration of antibiotic treatment to the development of antibiotic resistance.

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Summary

Mastitis is an inflammation of the udder caused by bacteria that invade through the teat canal. It is the most costly disease on a dairy farm because it directly affects the production of milk, the primary source of income for the dairy farmer. Mastitis goes along with visible symptoms in milk or udder (clinical mastitis) or is invisible (subclinical mastitis). For the cow, mastitis can be a painful disease, potentially affecting animal welfare and milk quality. In the perception of the dairy farmer, mastitis is mainly an annoying disease, disturbing the milking routine and requiring extra labor, and antibiotic treatment. Some invading bacteria may cause a chronic persistent intramammary infection, while others typically cause a short, transient infection. Although prevention of mastitis is preferred over treatment, mastitis as a disease cannot be eradicated and eventually, mastitis cases will occur and treatment is unavoidable. During treatment, farmers are uncertain whether the cow will ever return to full production. A large proportion of farmers repeats antibiotic treatment after initial treatment according the label claim, thereby extending the duration of treatment. The aim of the studies described in this thesis is to evaluate the bacteriological, clinical and economic efficacy of extended antibiotic treatment of persistent mastitis during lactation compared to standard treatment. An additional aim was to explore the social factors that influence farmers with respect to their choices on duration of antibiotic treatment of clinical mastitis.

In Chapter 2 and 3 we studied the economic effect, by means of partial budgeting, of lactational antibiotic treatment of persistent subclinical intramammary infections due to *Streptococcus uberis* or *Streptococcus dysgalactiae* (Chapter 2) and *Staphylococcus aureus* (Chapter 3). Effects at cow level and herd level were modeled, including prevention of clinical mastitis episodes and the prevention of transmission of infections.

On farms where pathogen transmission was prevented through proper udder health management, 3-day antibiotic treatment during lactation resulted in an average net profit of €11.62 for both streptococcal species, and a net loss of €-21.12 for *Staph. aureus.* In this optimal management situation, extended 8-day treatment had a more negative average net result for *Strep. uberis* or *Strep. dysgalactiae* (€-21.83) as well as for *Staph. aureus* (€-57.70).

On farms where pathogen transmission was high through suboptimal udder health management, as described in an outbreak situation for *Strep. uberis* or *Strep. Dysgalactiae,* where the Reproduction ratio (R) = 1.4, and for *Staph. aureus* where R = 5.3, 3-day treatment resulted in a net profit of €68.60 for both streptococcal species and €95.62 for *Staph. aureus*. In this high transmission situation, extended 8-day treatment increased the average benefit for *Staph. aureus* (€142.42), but decreased the average benefit for *Strep. uberis* or *Strep. dysgalactiae* (€58.62). The reason for this difference during the evaluated outbreaks is that the R-value of the *Staph. aureus* scenario was considerably higher than in the streptococci scenario, resulting in the prevention of more new cases after cure of *Staph. aureus*.

Sensitivity analysis in both, Chapter 2 and 3 showed that the 6 most influential input variables in the model were chance of bacteriological cure, the R-value, probability

of culling, retention pay-off, and cost of antibiotics and bacterial culture. Three-day antibiotic treatment of persistent subclinical *Strep. uberis* or *Strep. dysgalactiae* mastitis is economically profitable over a range of input values for cure probabilities, transmission rates and losses due to culling. For *Strep. uberis* and / or *Strep. dysgalactiae,* as well as for *Staph. aureus*, extended 8-day lactational treatment was found to be profitable only for very valuable animals, on farms where the risk of pathogen transmission is high and/or on farms where the farmer is likely to cull a high percentage of cows with subclinical mastitis. Treatment of subclinical mastitis is economically beneficial in many situations. However, because bacterial flora, cow characteristics and management differ widely between farms, the economic outcome of lactational treatment of chronic subclinical streptococcal and *Staph. aureus* mastitis is highly dependent on the farm, cow and pathogen involved.

In Chapter 4, bacteriological and clinical efficacy and evolution of quarter somatic cell count (SCC) after extended 5-day versus standard 1.5-day cefquinome treatment of clinical *Staph. aureus* mastitis was studied in a multi-centered, non-blinded, randomized clinical trial in 5 European countries; France, Hungary, Italy, the Netherlands, and the United Kingdom. Clinical cure was significantly better after extended treatment (82%) than after standard treatment (60%). This was, however, not confirmed by the bacteriological findings. Bacteriological cure rate after extended treatment (27%) was not better than after standard treatment (34%). The most important factor predicting bacteriological cure was pretreatment cow SCC. If cow SCC before treatment was higher than 250,000 cells/mL, bacteriological cure was less likely. The SCC of the affected clinical quarters after treatment was not different between treatment groups. Independent of the treatment regimen, bacteriologically cured quarters had a significantly lower SCC than non-bacteriologically cured quarters. Also, firm udders were less likely to cure clinically. It appeared that successful treatment (bacteriological cure) of clinical *Staph. aureus* mastitis with cefquinome is associated with an increased rate of new infections after treatment, with other pathogens (42%), mainly with coagulase negative staphylococci, as compared to non-cured quarters (22%). The total of the results indicate that extending treatment of clinical *Staph. aureus* mastitis with cefquinome should not be recommended.

In Chapter 5, a randomized, non-blinded, clinical field trial was performed to compare the efficacy of extended 5-day intramammary cefquinome treatment, a combination of this treatment regimen with an additional 5-day parenteral cefquinome treatment, and a standard 1.5-day intramammary cefquinome treatment of clinical mastitis. Treatment efficacy was evaluated independent of the pathogen, during a 105 day follow-up period of cases. The criteria for efficacy were persistence or recurrence of clinical signs, as this reflects farmers interpretation of a successful treatment in the field. The mastitis etiology was predominantly from an environmental origin (*Escherichia coli* and *Strep. uberis*) with a high rate of recurrence of clinical mastitis. Both, extended intramammary and the combination of extended intramammary and parenteral treatment, significantly decreased the persistence

or recurrence of clinical signs by 8% and 6% at the quarter level and by 9% and 8% at the cow level, respectively, compared to standard treatment. When intramammary treatment was extended, additional parenteral treatment did not further reduce the persistence or recurrence of clinical signs. As in Chapter 4, this chapter showed that extended cefquinome treatment regimens have an improved clinical outcome compared to standard treatment.

In Chapter 6, a randomized, non-blinded, clinical field trial was performed comparing the efficacy of an extended, 5-day intramammary cefquinome treatment with the efficacy of a standard, 1.5-day cefquinome treatment of clinical mastitis cases on 20 farms in Germany. Because extended treatment of clinical mastitis is frequently performed in cows with persistent high SCC, expecting a better cure, both treatment regimens were studied in clinical mastitis cases immediately preceded by at least two consecutive monthly cow level SCC > 200,000 cells/ml. The primary efficacy criteria were bacteriological and clinical cure, while quarter SCC cure was considered a secondary efficacy criterion. Overall bacteriological cure was not different after extended treatment (79%) or standard treatment (72%). Extended treatment, as compared to standard treatment, improved bacteriological cure when caused by streptococci, specifically *Strep. uberis*. At day 1.5 (36 hours after the start of treatment), only 13% of quarters showed clinical cure, increasing to 60% at day 5 (120 hours after the start of treatment), and 99%, both at day 14 and day 21. No significant difference in clinical cure was present between treatment groups. In our data, clinical cure after treatment was not related to eventual bacteriological cure. Overall quarter SCC cure was low (22%) and not significantly different between treatment groups. Overal quarter SCC cure, however, was significantly higher for cases due to Enterobacteriacae, as compared to staphylococci. Extended treatment with cefquinome of clinical mastitis in cows with a persistent high SCC seems to be only indicated when caused by streptococci, mainly *Strep. uberis*, but, on average, showed no advantage when the bacteriological cause of mastitis was not available.

In Chapter 7, a qualitative study, using semi-structured interviews, was performed to evaluate social factors of Dutch and German dairy farmers influencing decision making on the duration of antibiotic treatment of clinical mastitis. Of the 38 farmers interviewed, 30 reported to routinely and 7 to occasionally extend antibiotic treatment. Farmers' perception is that mastitis is not treated 'thoroughly' if clinical symptoms are still visible at the time of cessation of treatment. They believe that if mastitis is not treated thoroughly, it may persist or recur. Dairy farmers are sensitive towards social norms of other farmers and towards recognition by them for good stockmanship. Extended treatment seems to be part of the social norm of 'being a good farmer'. Specifically more 'cow-oriented' farmers expressed insecurity on how to treat mastitis effectively. This insecurity makes them more sensitive to comply to other farmers injunctive ('what ought to be') and descriptive ('what is done') norms. Additionally, they are influenced by the veterinarians informational norm that extended treatment is better, when applied resulting in an approved social norm. Social

approval reduces insecurity of potentially being perceived as a poor farmer and thus, extended treatment is emotionally rewarded.

Other farmers, the herd veterinarian, and other farm advisors were perceived by the interviewed farmers as positive reference groups, all regularly communicating face to face with them. Positive reference groups result in a positive judgment and have more influence towards socially accepted behavior than other groups. Society was the most negative reference group. The emotional gap between farmers and society is large and probably difficult to overcome. Society will therefore have little influence on the social norms of farmers. Evidence based information on treatment efficacy or practical criteria to decide on cure, may be able to change social norms of 'thorough' treatment, especially when communicated by a positive reference group such as veterinarians. Because prudent antibiotic use is hindered by perceived subjective norms on duration of antibiotic treatment, more research is needed, particularly, on optimal duration of treatment of clinical mastitis, caused by specific pathogens, in specific cows and with specific antibiotics, as related to cure and recurrence.

In Chapter 8, the previous chapters were discussed in the light of practical implications for the dairy farmer and the veterinary practitioner, under the current situation in the EU, and specifically in the Netherlands. The thesis showed that extending antibiotic treatment of persistent bovine mastitis during lactation is widely applied but is not always better than standard treatment with respect to bacteriological, clinical and economic efficacy. A higher efficacy of extended antibiotic treatment of persistent mastitis may be limited to specific antibiotics, cows, pathogens and farms. The farmers' decision to extend treatment was shown to be not only based on rational arguments, but mainly on subjective beliefs.

Efficacy of antibiotic treatment is influenced by preventive udder health management measures at least as much as by the duration of treatment. Thus, veterinarians and farmers should give attention to udder health management aspects rather than on (extended) treatment protocols alone. Nevertheless, when a mastitis case is treated, at some point in time, it has to be decided whether to stop or to extend a treatment. Therefore, there is a need in the field for a tool to decide on the optimal duration of treatment of mastitis from the perspective of bacteriological, clinical and economic efficacy. Also, the relationship between duration of treatment and the consequences for antibiotic resistance development is not clear and currently, arguments in either direction can be made. Thus, future research should focus on developing reliable, cheap and easy to perform, preferably cow-side, tests to determine the optimal moment when to stop treatment. Additionally, there is a need for quantitative studies that relate duration of antibiotic treatment to the development of antibiotic resistance.

Samenvatting

Mastitis is een ontsteking van de uier veroorzaakt door bacteriën die binnendringen via het tepelkanaal. Het is de meest kostbare ziekte op een melkveebedrijf omdat het direct effect heeft op de melkproductie, de belangrijkste inkomstenbron van de melkveehouder. Mastitis kan zichtbare symptomen laten zien in melk of uier (klinische mastitis), maar kan ook zonder zichtbare symptomen verlopen (subklinische mastitis). Voor de koe kan mastitis pijnlijk zijn. Mastitis kan dus het dierenwelzijn en de melkkwaliteit aantasten. Voor de veehouder is mastitis vooral een vervelende aandoening die de dagelijkse routine van het melken verstoort, extra arbeid kost en behandeld moet worden met antibiotica. Sommige binnendringende bacteriën kunnen een persistente infectie veroorzaken, terwijl andere tot een korte, tijdelijke infectie leiden. Hoewel het voorkómen van mastitis de voorkeur heeft boven de behandeling ervan, zal preventie de ziekte mastitis nooit helemaal doen verdwijnen, zullen klinische gevallen dus blijven optreden en is behandeling daarvan onvermijdelijk. Ten aanzien van de behandeling van mastitis zijn veehouders vooral onzeker of de koe wel weer volledig in productie zal komen. Een groot deel van de veehouders herhaalt de antibioticumbehandeling na uitvoering van de initiële standaardbehandeling volgens de bijsluiter, waardoor de behandelingsduur verlengd wordt. Het doel van de onderzoeken beschreven in dit proefschrift is de bacteriologische, klinische en economische effecten van een verlengde antibioticumbehandeling van persisterende mastitis gedurende de lactatie te vergelijken met die van de standaardbehandeling. Een aanvullend doel was om de sociale factoren die van invloed zijn op de keuzes die veehouders maken ten aanzien van de duur van de antibioticumbehandeling van klinische mastitis in beeld te brengen en te analyseren.

In hoofdstuk 2 en 3 is het economische effect, van een antibioticumbehandeling gedurende de lactatie van een persistente subklinische uierinfectie, veroorzaakt door *Streptococcus uberis* of *Streptococcus dysgalactiae* (hoofdstuk 2) en *Staphylococcus aureus* (hoofdstuk 3), met behulp van partial budgeting bestudeerd. Effecten op koe- en koppel niveau zijn gemodelleerd, inclusief het voorkomen van klinische opflikkeringen en de overdracht van infecties.

Op bedrijven waar door goed uiergezondheidsmanagement de overdracht van pathogenen wordt voorkomen, resulteert een 3-daagse antibioticumbehandeling gedurende de lactatie in een netto opbrengst van €11.62 voor beide streptococcensoorten en een netto kostenpost van €21.12 voor *Staph. aureus.* In deze optimale situatie leidde een verlengde 8-daagse behandeling tot een negatiever netto resultaat, zowel voor *Strep. uberis* of *Strep. dysgalactiae* (€-21.83) als voor *Staph. aureus* (€-57.70), dan een 3-daagse behandeling.

Op melkveebedrijven waar door suboptimaal uiergezondheidsmanagement de overdracht van pathogenen hoog was, zoals beschreven voor een uitbraak van *Strep. uberis* of *Strep. dysgalactiae*, met een Reproductie ratio (R) van 1.4 en voor *Staph. aureus* met een R van 5.3, resulteerde een 3-daagse behandeling in een netto opbrengst van €68.60 voor beide streptococcensoorten en van €95.62 voor *Staph. aureus*. In deze omstandigheden van hoge overdracht, verhoogde het verlengen van een behandeling van *Staph. aureus* tot 8 dagen de gemiddelde opbrengst naar €142.42, maar verminderde de gemiddelde opbrengst van een *Strep. uberis* of *Strep. dysgalactiae* behandeling naar €58.62. De

reden van dit verschil tijdens de beschreven uitbraken is dat de R-waarde in het *Staph. aureus* - scenario veel hoger was dan die in het streptococcenscenario, wat resulteerde in het voorkómen van meer nieuwe uierinfecties na de genezing van *Staph. aureus*.

De gevoeligheidsanalyse in hoofdstuk 2 en 3 laat zien dat de 6 belangrijkste input-variabelen in het model zijn: de kans op bacteriologische genezing, de R-waarde, de kans op afvoer, de winst van het aanhouden van de koe ten opzichte van afvoeren en de kosten van antibiotica en bacteriologisch onderzoek. Een 3-daagse antibioticumbehandeling van persisterende subklinische *Strep. uberis* of *Strep. dysgalactiae* mastitis, blijkt voor een hele range van inputvariabelen economisch aantrekkelijker te zijn dan een 8-daagse behandeling. Voor zowel *Strep. uberis* en *Strep. dysgalactiae,* als voor *Staph. aureus*, was een verlengde 8-daagse lactatiebehandeling alleen winstgevend voor zeer waardevolle koeien, op bedrijven waar de kans op overdracht van pathogenen hoog is en/of op bedrijven waar de afvoer van koeien met subklinische mastitis hoog is.

Behandeling van subklinische mastitis is economisch rendabel onder veel omstandigheden. Maar, omdat bacteriële flora, koe-factoren en management sterk verschillen tussen bedrijven, is de economische uitkomst van een behandeling van persisterende subklinische *Strep. uberis* of *Strep. dysgalactiae* en *Staph. aureus* mastitis tijdens de lactatie erg afhankelijk van het bedrijf, de koe en de betrokken pathogeen.

In hoofdstuk 4 is de bacteriologische en klinische werkzaamheid en het verloop van het kwartier-celgetal beschreven na een verlengde, 5-daagse, behandeling en na een standaard 1.5-daagse behandeling van klinische *Staph. aureus* mastitis met cefquinome. Dit is geëvalueerd in een niet geblindeerd, gerandomiseerd klinische veldonderzoek in 5 Europese landen: Frankrijk, Hongarije, Italië, Nederland en Groot-Brittannië. De klinische genezing na een verlengde behandeling (82%) was significant beter dan na een standaardbehandeling (60%). Dit werd echter niet ondersteund door de bacteriologische bevindingen. De bacteriologische genezing na verlengde behandeling (27%) was niet significant beter dan na een standaardbehandeling (34%). De belangrijkste voorspellende factor voor bacteriologische genezing was niet de lengte van de behandeling, maar de hoogte van het koe-celgetal vóór de behandeling. Als dat celgetal hoger was dan 250,000 cells/mL, was de kans op bacteriologische genezing na behandeling aanmerkelijk lager. Het celgetal van het behandelde kwartier was na behandeling niet significant verschillend voor de 2 behandelingsgroepen. Onafhankelijk van de behandeling, hadden bacteriologisch genezen kwartieren een significant lager celgetal dan kwartieren die niet genezen waren. Ook hadden harde uiers een significant lagere kans om klinisch te genezen. Het lijkt erop dat een succesvolle behandeling (bacteriologische genezing) van klinische *Staph. aureus* mastitis met cefquinome samengaat met een hoger percentage nieuwe infecties met andere pathogenen (42%), voornamelijk coagulase negatieve staphylococci, dan bij niet genezen kwartieren (22%). Het totaal van de resultaten geeft aan dat een verlengde behandeling van klinische *Staph. aureus* mastitis niet standaard geadviseerd zou moeten worden.

In hoofdstuk 5 is een gerandomiseerd, niet-blind, klinisch veldonderzoek beschreven, waarin een verlengde, 5-daagse, cefquinome behandeling in de uier, een combinatie van deze behandeling met een aanvullende, 5-daagse, parenterale toediening met cefquinome en een standaard, 1.5-daagse, cefquinome behandeling van klinische mastitis zijn vergeleken. De werkzaamheid van de behandeling is gedurende een 105-daagse follow-up periode van klinische mastitisgevallen, onafhankelijk van de oorzakelijke pathogeen geëvalueerd. De criteria voor werkzaamheid waren het aanhouden of het weer terugkomen van klinische symptomen na de behandeling, omdat dit voor de veehouder de belangrijkste parameter voor een succesvolle behandeling is. De mastitisverwekkers waren voornamelijk 'omgevingskiemen' (*Escherichia coli* en *Strep. uberis*) waarbij een groot aantal mastitisgevallen met intermitterende klinische symptomen voorkwam. Zowel de verlengde behandeling in de uier als de combinatie hiervan met parenterale behandeling, leidden tot een significante verlaging van het aanhouden of weer terugkomen van klinische symptomen na behandeling, met respectievelijk 8% en 6% op kwartierniveau en met respectievelijk 9% en 8% op koeniveau, in vergelijking met standaardbehandeling. In vergelijking met de verlengde behandeling in de uier, leidde de aanvullende parenterale behandeling niet tot een verdere verbetering van het klinisch herstel. Net zoals hoofdstuk 4, laat dit hoofdstuk zien dat beide verlengde cefquinome behandelingregimes een betere klinische uitkomst hebben dan standaardbehandeling.

Hoofdstuk 6 beschrijft een gerandomiseerd, niet geblindeerd, klinisch veldonderzoek op 20 melkveebedrijven in Duitsland. In deze proef werd een 5-daagse behandeling van klinische mastitis in de uier vergeleken met een 1.5-daagse behandeling. Klinische mastitis bij koeien met een aanhoudend hoog celgetal wordt vaak verlengd behandeld omdat dan een hogere genezing verwacht wordt. Om die reden werden beide klinische mastitisbehandelingen bestudeerd in koeien die voorafgaand aan het klinische mastitisgeval tenminste 2 opeenvolgende maandelijkse koe-celgetallen hadden van meer dan 200,000 cellen/ml. De primaire criteria voor werkzaamheid waren bacteriologische en klinische genezing, terwijl de verbetering van het kwartier-celgetal als een secundair criterium beschouwd werd. De bacteriologische genezing na verlengde behandeling (79%) bleek niet te verschillen van die van standaardbehandeling (72%). Verlengde behandeling verbeterde, in vergelijking met standaardbehandeling, echter wel de bacteriologische genezing van streptococcen, met name van *Strep. uberis*. Op 1.5 dag, 36 uur na de eerste behandeling, was 13% van de kwartieren klinisch genezen, toenemend naar 60% op dag 5, 120 uur na de eerste behandeling, en naar 99% op zowel dag 14 als dag 21. Er werd echter geen significant verschil gevonden in klinische genezing tussen de behandelgroepen. Volgens onze data was er geen relatie tussen de klinische genezing na behandeling en de uiteindelijke bacteriologische genezing. Slechts in 22% van de gevallen trad na behandeling een verlaging van het kwartier-celgetal tot onder de 200.000 cellen/ml op, niet significant verschillend tussen de behandelgroepen. Deze verbetering van het kwartier-celgetal was echter significant hoger voor mastitisgevallen veroorzaakt door Enterobacteriacae

dan voor mastitisgevallen veroorzaakt door staphylococcen. Een verlengde cefquinome behandeling van klinische mastitiskoeien met aanhoudend hoog celgetal lijkt alleen zinvol als deze veroorzaakt worden door streptococcen, met name door *Strep. uberis*. Gemiddeld genomen echter, als de mastitisverwekker niet bekend is, biedt een verlengde behandeling geen voordelen.

In hoofdstuk 7 is een kwalitatief onderzoek beschreven, waarin semi-gestructureerde interviews zijn gebruikt om de sociale invloeden op Duitse en Nederlandse melkveehouders, in relatie tot de duur van de antibioticumbehandeling, in beeld te brengen. Van de 38 geïnterviewde veehouders, gaven 30 aan dat ze routinematig, en 7 dat ze af en toe de antibioticumbehandeling verlengden. De melkveehouders vonden dat mastitis niet 'grondig' behandeld is, als na de laatste behandeling nog klinische symptomen zichtbaar zijn. Bovendien geloven zij dat als mastitis niet grondig behandeld wordt, de kans groot is dat de symptomen aanhouden of terugkomen. Melkveehouders blijken gevoelig voor sociale normen van andere melkveehouders en voor het gevoel door hen mogelijk niet als een goede boer gezien te worden. Het langer behandelen van klinische mastitis lijkt een onderdeel te zijn van de sociale norm voor 'een goede boer'. Vooral de meer 'koe-georiënteerde' melkveehouders, gaven duidelijk aan dat ze onzeker zijn over hoe ze mastitis goed moeten behandelen. Deze onzekerheid maakt dat ze graag willen voldoen aan de injunctieve ('hoe hoort het') en de beschrijvende ('wat wordt gedaan') normen van andere melkveehouders. Bovendien worden ze beïnvloed door de informatieve norm van de dierenarts dat verlengde behandeling beter is, waarmee de sociale norm bevestigd wordt. Het bevestigen van de sociale norm vermindert het risico om gezien te worden als een slechte boer en dus wordt verlengde behandeling emotioneel beloond.

Andere melkveehouders, de eigen dierenarts en andere bedrijfsadviseurs hadden allemaal regelmatig persoonlijk contact met de geïnterviewde melkveehouders en werden door hen ervaren als een positieve referentiegroep. Positieve referentiegroepen worden positief beoordeeld en hebben meer invloed op sociaal geaccepteerd gedrag dan andere groepen. De maatschappij was de meest negatieve referentiegroep. Het emotionele gat tussen melkveehouders en de maatschappij is groot en waarschijnlijk moeilijk te overbruggen. De maatschappij zal daarom weinig invloed hebben op de sociale normen van veehouders. Wetenschappelijk onderbouwde informatie over de werkzaamheid van behandeling, of praktische criteria om genezing vast te stellen, zijn misschien in staat om de sociale norm van 'grondig' behandelen te veranderen, vooral als deze gecommuniceerd wordt door een positieve referentiegroep zoals bijvoorbeeld de dierenarts. Verantwoord gebruik van antibiotica wordt belemmerd door subjectieve normen over de duur van de behandeling. Daarom is meer onderzoek nodig naar de optimale behandelingsduur in relatie tot genezing en herhalingsgevallen van klinische mastitis veroorzaakt door specifieke pathogenen, in specifieke koeien en met specifieke antibiotica.

In Hoofdstuk 8 worden de voorgaande hoofstukken bediscussieerd in relatie tot de praktische gevolgen voor de melkveehouder en de praktiserende dierenarts, onder de huidige regelgeving in de EU, in het bijzonder in Nederland. Dit proefschrift laat zien dat een verlengde antibioticumbehandeling van persisterende mastitis gedurende de lactatie veelvuldig wordt toegepast, maar dat de bacteriologische, klinische en economische werkzaamheid niet altijd beter is dan die van een standaardbehandeling. Een hogere werkzaamheid van een verlengde antibioticumbehandeling lijkt beperkt tot specifieke melkveebedrijven, koeien, pathogenen en antibiotica. Het bleek dat beslissingen van melkveehouders om een klinische mastitisbehandeling al of niet te verlengen niet alleen gebaseerd zijn op rationele argumenten, maar dat ook subjectieve meningen een rol spelen. De werkzaamheid van een antibioticumbehandeling wordt minstens zo veel beïnvloed door een effectief preventief uiergezondheidsmanagement als door de duur van de behandeling. Daarom zouden dierenartsen en melkveehouders ook aandacht moeten geven aan preventief uiergezondheidsmanagement en niet alleen aan (verlengde) behandelingsprotocollen. Desalniettemin moet bij ieder behandeld mastitisgeval op enig moment beslist worden om de behandeling te stoppen of voort te zetten. Daarom bestaat in de praktijk een behoefte aan hulpmiddelen die helpen om de optimale behandelingsduur met betrekking tot bacteriologische, klinische en economische werkzaamheid vast te stellen. De relatie tussen de duur van de behandeling en de ontwikkeling van antibioticumresistentie is niet duidelijk, omdat op dit moment beide kanten op geredeneerd kan worden. Toekomstig onderzoek zou zich dus moeten richten op de ontwikkeling van goedkope, betrouwbare en gemakkelijk uit te voeren diagnostiek, bij voorkeur uit te voeren naast de koe, die het optimale moment vaststelt waarop een behandeling gestopt kan worden. Daarnaast is er behoefte aan kwantitatief onderzoek dat de relatie tussen de duur van de behandeling en de ontwikkeling van antibioticumresistentie onderzoekt.

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Curriculum vitae
Biography

Curriculum vitae

Jantijn Swinkels werd geboren op 21 juli 1961 te Helmond. Na het afronden van het VWO op het Carolus Borromeus College te Helmond, werd aansluitend begonnen met de studie Diergeneeskunde in Utrecht, die in 1990 afgesloten werd met de differentiatie Landbouwhuisdieren. Tijdens de studie was hij bestuurlijk actief in het Veterinair Dispuut Unitas en de Diergeneeskundige Studentenkring en volgde hij de tropencursus, die afgesloten werd met een stage van 6 maanden in de Franse Antillen (Guadeloupe). Na 2 jaar gewerkt te hebben bij de toenmalige vakgroep Bedrijfsdiergeneeskunde en Buitenpraktijk van de Faculteit Diergeneeskunde, ging hij de praktijk in. In 1993 volgde een associatie in dierenartsenpraktijk Volendam/Edam/Oosthuizen (VEO), waarin hij zich, naast gezelschapsdieren en schapen, voornamelijk bezighield met de advisering van melkveebedrijven. Naast het praktijkwerk deed hij consultancy op het gebied van uiergezondheid, eerst voor Leo Pharma b.v en later voor Boehringer Ingelheim b.v.. In 2005 werd hij door de KNMvD erkend als Specialist Rundergezondheid. In datzelfde jaar begon hij bij Intervet b.v. in Boxmeer, later MSD Animal Health, als Global Technical Director, met als belangrijkste aandachtspunten uiergezondheid en luchtwegaandoeningen. Toen eind 2012 het hoofdkantoor van MSD Animal Health naar de Verenigde Staten verhuisde, ging hij naar de Gezondheidsdienst voor Dieren in Deventer, waar hij nu verantwoordelijk is voor het uiergezondheidsteam.

Biography

Jantijn Swinkels was born on July 21, 1961 in Helmond, The Netherlands. After graduating from secondary school at the Carolus Borromeus College te Helmond, he started to study Veterinary Medicine at the University of Utrecht, where he graduated in 1990, specialized in Food Animal Medicine. During his studies, he followed the course Tropical Veterinary Medicine, including an internship in the French West Indies (Guadeloupe). After 2 years at the ambulatory clinic of the Bovine Herd Health department of the Faculty of Veterinary Medicine, he joined practice. In 1993, he became a partner at the veterinary practice Volendam/Edam/Oosthuizen (VEO), in which he, besides companion animals and sheep, mainly focused on herd health consultancy in dairy farms. During the years in practice, he did consultancy on udder health, initially for Leo Pharma b.v., later for Boehringer Ingelheim b.v.. In 2005 he was acknowledged as Bovine Herd Health Specialist, by the Dutch Veterinary Association. In the same year he joined Intervet b.v., that later became MSD Animal Health as a Global Technical Director, mainly focusing on udder and lung health. When the head quarters of MSD Animal Health b.v. were relocated to the US, he joined GD Animal Health in Deventer, where he is now responsible for the udder health team.

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